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(54) Title: RECOMBINANT SWINEPOX VIRUS (57) Abstract This invention provides a recombinant swinepox virus comprising a foreign DNA inserted into a swinepox virus genome, wherein the foreign DNA is inserted into a) an <i>AccI</i> site within a region corresponding to a 3.2 Kb <i>HindIII</i> to <i>BglII</i> subfragment of the <i>HindII</i> M fragment and b) an <i>EcoRI</i> site within a region corresponding to a 3.2 Kb subfragment of the <i>HindIII</i> K fragment which contains both a <i>HindIII</i> and <i>EcoRI</i> site, of the swinepox virus genome and is capable of being expressed in a host cell into which the virus is introduced. The invention further provides vaccines and methods of immunization of the recombinant swinepox virus.		

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RECOMBINANT SWINEPOX VIRUS

5 Within this application several publications are
referenced by arabic numerals within parentheses. Full
citations for these publications may be found at the end
of the specification immediately preceding the claims.
The disclosures of these publications are hereby
10 incorporated by reference into this application.

BACKGROUND OF THE INVENTION

Swinepox virus (SPV) belongs to the family Poxviridae.
15 Viruses belonging to this group are large, double-
stranded DNA viruses that characteristically develop in
the cytoplasm of the host cell. SPV is the only member
of the genus Suipoxvirus. Several features distinguish
SPV from other poxviruses. SPV exhibits species
20 specificity (18) compared to other poxviruses such as
vaccinia which exhibit a broad host range. SPV infection
of tissue culture cell lines also differs dramatically
from other poxviruses (24). It has also been
demonstrated that SPV does not exhibit antigenic cross-
25 reactivity with vaccinia virus and shows no gross
detectable homology at the DNA level with the ortho,
lepori, avi or entomopox virus groups (24). Accordingly,
what is known and described in the prior art regarding
other poxviruses does not pertain a priori to swinepox
30 virus.

SPV is only mildly pathogenic, being characterized by a
self-limiting infection with lesions detected only in the
skin and regional lymph nodes. Although the SPV
35 infection is quite limited, pigs which have recovered
from SPV are refractory to challenge with SPV, indicating
development of active immunity (18).

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The present invention concerns the use of SPV as a vector for the delivery of vaccine antigens and therapeutic agents to swine. The following properties of SPV support this rationale: SPV is only mildly pathogenic in swine, SPV is species specific, and SPV elicits a protective immune response. Accordingly, SPV is an excellent candidate for a viral vector delivery system, having little intrinsic risk which must be balanced against the benefit contributed by the vector's vaccine and therapeutic properties.

The prior art for this invention stems first from the ability to clone and analyze DNA while in bacterial plasmids. The techniques that are available are detailed for the most part in Maniatis et al., 1983 and Sambrook et al., 1989. These publications teach state of the art general recombinant DNA techniques.

Among the poxviruses, five (vaccinia, fowlpox, canarypox, pigeon, and raccoon pox) have been engineered, previous to this disclosure, to contain foreign DNA sequences. Vaccinia virus has been used extensively to vector foreign genes (25) and is the subject of U.S. Patents 4,603,112 and 4,722,848. Similarly, fowlpox has been used to vector foreign genes and is the subject of several patent applications EPA 0 284 416, PCT WO 89/03429, and PCT WO 89/12684. Raccoon pox (10) and Canarypox (31) have been utilized to express antigens from the rabies virus. These examples of insertions of foreign genes into poxviruses do not include an example from the genus *Suipoxvirus*. Thus, they do not teach methods to genetically engineer swinepox viruses, that is, where to make insertions and how to get expression in swinepox virus.

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The idea of using live viruses as delivery systems for antigens has a very long history going back to the first live virus vaccines. The antigens delivered were not foreign but were naturally expressed by the live virus in the vaccines. The use of viruses to deliver foreign antigens in the modern sense became obvious with the recombinant vaccinia virus studies. The vaccinia virus was the vector and various antigens from other disease causing viruses were the foreign antigens, and the vaccine was created by genetic engineering. While the concept became obvious with these disclosures, what was not obvious was the answer to a more practical question of what makes the best candidate virus vector. In answering this question, details of the pathogenicity of the virus, its site of replication, the kind of immune response it elicits, the potential it has to express foreign antigens, its suitability for genetic engineering, its probability of being licensed by regulatory agencies, etc, are all factors in the selection. The prior art does not teach these questions of utility.

The prior art relating to the use of poxviruses to deliver therapeutic agents relates to the use of a vaccinia virus to deliver interleukin-2 (12). In this case, although the interleukin-2 had an attenuating effect on the vaccinia vector, the host did not demonstrate any therapeutic benefit.

The therapeutic agent that is delivered by a viral vector of the present invention must be a biological molecule that is a by-product of swinepox virus replication. This limits the therapeutic agent in the first analysis to either DNA, RNA or protein. There are examples of therapeutic agents from each of these classes of compounds in the form of anti-sense DNA, anti-sense RNA (16), ribozymes (34), suppressor tRNAs (2), interferon-

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inducing double stranded RNA and numerous examples of protein therapeutics, from hormones, e.g., insulin, to lymphokines, e.g., interferons and interleukins, to natural opiates. The discovery of these therapeutic
5 agents and the elucidation of their structure and function does not make obvious the ability to use them in a viral vector delivery system.

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SUMMARY OF THE INVENTION

This invention provides a recombinant swinepox virus comprising a foreign DNA inserted into a swinepox virus genome, wherein the foreign DNA is inserted into an EcoRI
5 site within a region corresponding to a 3.2 Kb subfragment of the HindIII K fragment which contains both a HindIII and an EcoRI site, of the swinepox virus genome and is capable of being expressed in a host cell into which the virus is introduced.

10

This invention provides a recombinant swinepox virus comprising a foreign DNA inserted into a swinepox virus genome, wherein the foreign DNA is inserted into a) an
15 AccI site within a region corresponding to a HindIII to BglII subfragment of the HindIII M fragment and b) an EcoRI site within a region corresponding to a 3.2 Kb subfragment of the HindIII K fragment which contains both a HindIII and EcoRI site, of the swinepox virus genome and is capable of being expressed in a host cell into
20 which the virus is introduced.

The invention further provides vaccines and methods of immunization of the recombinant swinepox virus.

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BRIEF DESCRIPTION OF THE INVENTION**Figures 1A - 1B:**

5 Show a detailed diagram of SPV genomic DNA (Kasza strain) including the unique long and Terminal repeat (TR) regions. A restriction map for the enzyme *HindIII* is indicated (23). Fragments are lettered in order of decreasing size. Note that the terminal repeats are greater than 2.1 kb but less than 9.7 kb in size.

10

Figures 2A - 2C:

15 Show the homology which exists between the 515.85.1 ORF and the Vaccinia virus 01L ORF. Figure 2A shows two maps: The first line of Figure 2A is a restriction map of the SPV *HindIII* M fragment and the second is a restriction map of the DNA insertion in plasmid 515-85.1. The location of the 515-85.1 [VV 01L-like] ORF is also indicated on the map. The locations of the DNA sequences shown in Figures 2B and 2C are indicated below the map by heavy bars in Figure 2A. Figure 2B shows the homology between the VV 01L ORF and the 515-85.1 ORF at their respective N-termini. Figure 2C shows the homology between the VV 01L ORF and the 515-85.1 ORF at their respective C-termini.

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Figures 3A - 3C:

30 Show a description of the DNA insertion in Homology Vector 520-17.5. Figure 3A contains a diagram showing the orientation of DNA fragments assembled in plasmid 520-17.5 and table indicating the origin of each fragment. Figure 3B shows the sequences located at each of the junctions A and B between fragments, and Figure 3C shows the sequences located at Junctions C and D. Figures 3B and 3C further describe the restriction sites used to generate each

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fragment as well as the synthetic linker sequences which were used to join the fragments are described for each junction. The synthetic linker sequences are underlined by a heavy bar. The location of several gene coding regions and regulatory elements are also given. The following two conventions are used: numbers in parenthesis () refer to amino acids, and restriction sites in brackets [] indicate the remnants of sites which were destroyed during construction. The following abbreviations are used, swinepox virus (SPV), early promoter 1 (EP1), late promoter 2 (LP2), lactose operon Z gene (lacZ), and *Escherichia coli* (*E. coli*).

15 Figures 4A- 4D:

Show a detailed description of the DNA insertion in Homology Vector 538-46.16. Figure 4A contains a diagram showing the orientation of DNA fragments assembled in plasmid 538-46.16 and a table indicating the origin of each fragment. Figure 4B shows the sequences located at Junctions A and B between fragments, Figure 4C shows sequences located at Junction C and Figure 4D shows sequences located at Junctions D and E. Figures 4B to 4D also describe the restriction sites used to generate each fragment as well as the synthetic linker sequences which were used to join the fragments are described for each junction. The synthetic linker sequences are underlined by a heavy bar. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parenthesis () refer to amino acids, and restriction sites in brackets [] indicate the remnants of sites which were destroyed during construction. The following abbreviations are used, swinepox virus (SPV), pseudorabies virus (PRV), g50 (gD), glycoprotein 63 (g63), early promoter 1 (EP1),

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late promoter 1 (LP1), late promoter 2 (LP2), lactose operon Z gene (lacZ), and *Escherichia coli* (*E. coli*).

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Figures 5A - 5D:

10 Show a detailed description of Swinepox Virus S-PRV-013 and the DNA insertion in Homology Vector 570-91.64. Figure 5A contains a diagram showing the orientation of DNA fragments assembled in plasmid 570-91.64 and a table indicating the origin of each fragment. Figure 5B shows the sequences located at Junctions A and B between fragments, Figure 5C shows the sequences located at Junction C, and Figure 5D shows the sequences located at Junctions D and E. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction in Figures 5B to 5D. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), *Escherichia coli* (*E. coli*), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2), gIII (gC) base pairs (BP).

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Figure 6:

35 Map showing the 5.6 kilobase pair HindIII M swinepox virus genomic DNA fragment. Open reading frames (ORF) are shown with the number of amino acids coding in each open reading frame. The swinepox virus ORFs show significant sequence identities to

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the vaccinia virus ORFs and are labeled with the vaccinia virus nomenclature (56 and 58). The I4L ORF (SEQ ID NO: 196) shows amino acid sequence homology to ribonucleotide reductase large subunit (57), and the O1L ORF (SEQ ID NO: 193) shows amino acid sequence homology to a leucine zipper motif characteristic of certain eukaryotic transcriptional regulatory proteins (13). The BglIII site in the I4L ORF and the AccI site in the O1L ORF are insertion sites for foreign DNA into non-essential regions of the swinepox genome. The homology vector 738-94.4 contains a deletion of SPV DNA from nucleotides 1679 to 2452 (SEQ ID NO: 189). The black bar at the bottom indicates regions for which the DNA sequence is known and references the SEQ ID NOs: 189 and 195. Positions of restriction sites AccI, BglIII, and HindIII are shown. I3L ORF (SEQ ID NO: 190), I2L ORF (SEQ ID NO: 191) and E1OR ORF (SEQ ID NO: 194) are shown. SEQ ID NO 221 contains the complete 5785 base pair sequence of the HindIII M fragment. Open reading frames within the SPV HindIII M fragment are the partial I4L ORF (445 AA; Nucl 2 to 1336); I3L ORF (275 AA; Nucl 1387 to 2211); I2L ORF (75 AA; Nucl 2215 to 2439); I1L ORF (313 AA; Nucl 2443 to 3381); O1L ORF (677 AA; Ncl 3520 to 5550); partial E1OR ORF (64 AA; Nucl 5787 to 5596).

Figures 7A - 7D:

Show a detailed description of Swinepox Virus S-SPV-015 and the DNA insertion in Homology Vector 727-54.60. Figure 7A contains a diagram showing the orientation of DNA fragments assembled in plasmid 727-54.60 and a table indicating the origin of each fragment. Figure 7B shows the sequences located at Junctions A and B between fragments, Figure 7C shows the sequences located at Junction C, and Figure 7D shows the sequences located at Junctions D and E.

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5 The restriction sites used to generate each fragment
 as well as synthetic linker sequences which are used
 to join the fragments are described for each
 junction in Figures 7B to 7D. The location of
 several gene coding regions and regulatory elements
 is also given. The following two conventions are
 used: numbers in parentheses, (), refer to amino
 acids, and restriction sites in brackets, [],
 10 indicate the remnants of sites which are destroyed
 during construction. The following abbreviations
 are used: swinepox virus (SPV), pseudorabies virus
 (PRV), *Escherichia coli* (*E. coli*), pox synthetic
 late promoter 1 (LP1), pox synthetic late promoter
 2 early promoter 2 (LP2EP2), glycoprotein B (gB),
 15 base pairs (BP).

Figures 8A-8D:

20 Detailed description of Swinepox Virus S-SPV-042 and
 the DNA insertion in Homology Vector 751-07.A1.
 Diagram showing the orientation of DNA fragments
 assembled in plasmid 751-07.A1. The origin of each
 fragment is indicated in the table. The sequence
 located at each of the junctions between fragments
 25 is also shown. The restriction sites used to
 generate each fragment as well as synthetic linker
 sequences which are used to join the fragments are
 described for each junction. Figures 8A-8D show the
 sequences located at Junction A (SEQ ID NOS: 197),
 30 (SEQ ID NO: 198), C (SEQ ID NO: 199), D (SEQ ID NO:
 200) and E (SEQ ID NO: 201) between fragments and
 the sequences located at the junctions. The
 location of several gene coding regions and
 regulatory elements is also given. The following
 35 two conventions are used: numbers in parentheses,
 (), refer to amino acids, and restriction sites in
 brackets, [], indicate the remnants of sites which

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are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), chicken myelomonocytic growth factor (cMGF), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2), polymerase chain reaction (PCR), base pairs (BP).

Figures 9A-9D:

Detailed description of Swinepox Virus S-SPV-043 and the DNA insertion in Homology Vector 751-56.A1. Diagram showing the orientation of DNA fragments assembled in plasmid 751-56.A1. The origin of each fragment is indicated in the table. The sequences located at each of the junctions between fragments is also shown. Figures 9A-9D show the sequences located at Junction A (SEQ ID NOS: 202), (SEQ ID NO: 203), C (SEQ ID NO: 204), D (SEQ ID NO: 205) and E (SEQ ID NO: 206) between fragments and the sequences located at the junctions. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), chicken interferon (cIFN), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LPE2EP2), polymerase chain reaction (PCR), base pairs (BP).

Figure 10A-10D:

Detailed description of Swinepox Virus S-SPV-037 and the DNA insertion in Homology Vector 752-22.1.

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Diagram showing the orientation of DNA fragments assembled in plasmid 752-22.1. The origin of each fragment is indicated in the table. The sequences located at each of the junctions between fragments is also shown. Figures 10A-10D show the sequences located at Junction A (SEQ ID NOS: 207), (SEQ ID NO: 208), C (SEQ ID NO: 209), and D (SEQ ID NO: 210) between fragments and the sequences located at the junctions. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restrictions sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), Escherichia coli (E. coli), pox synthetic late promoter 2 early promoter 2 (LP2EP2), polymerase chain reaction (PCR), base pairs (BP).

Figures 11A-11B:

Figure 11A: Restriction Endonuclease Map and Open Reading Frames in the SPV HindIII N fragment and part of SPV HindIII M fragment. Insertions of a foreign gene into a non-essential site of the swinepox virus Hind III N and Hind III M genomic DNA include the EcoR V site (S-SPV-060), SnaB I site (S-SPV-061), Bgl II site in Hind III N (S-SPV-062), and the Bgl II site in Hind III M (S-SPV-047). Insertions of a foreign gene into the I7L ORF (SEQ ID NO. 230) and I4L ORF (SEQ ID NO. 231) indicates that the sequence of the entire open reading frame is non-essential for replication of the swinepox virus and suitable for insertion of foreign genes.

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Additional sites for insertion of foreign genes include, but are not limited to the two Hind III sites, Ava I site, and the BamHI site.

5 Figure 11B: Restriction Endonuclease Map and Open
Reading Frames in the SPV Hind III K genomic
fragment. Insertion of a foreign gene into a non-
essential site of the swinepox virus Hind III K
10 genomic DNA includes, but is not limited to the
unique EcoR I site (S-SPV-059). Three open reading
frames (ORFs) were identified within an
approximately 3.2 kB region (SEQ ID NO. 1) of the
approximately 6.7 kb SPV HindIII K fragment.
15 Insertions of a foreign DNA into a unique EcoRI site
within the SPV HindIII K genomic fragment indicates
that the sequence is non-essential for replication
of the swinepox virus and suitable for insertion of
foreign genes. The unique EcoRI site is located
20 between the 77.2 kd protein ORF and the T5 protein
ORF in an intergenic region indicating that the
intergenic region contains suitable sites for
insertion of foreign DNA. Also identified are the
77.2 kd protein ORF (SEQ ID NO:3) and the T5 protein
25 ORF (SEQ ID NO. 4) and an ORF of unknown function
(SEQ ID NO. 2) which are suitable sites for
insertion of a foreign DNA. The SPV 77.2 kd protein
ORF (SEQ ID NO. 3) has amino acid sequence homology
to rabbit fibroma virus (RFV) 77.2 kd protein. The
30 SPV T5 protein ORF has amino acid sequence homology
to rabbit fibroma virus (RFV) T5 protein. The
identified open reading frames are within an
approximately 3141 base pair segment of the SPV Hind
III K fragment (SEQ ID NO. 1). The remaining
35 approximately 3500 base pairs of the SPV Hind III K
fragment has been sequenced previously (R.F.
Massung, et al. Virology 197, 511-528 (1993)).

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Figures 12A-12C:

Detailed description of Swinepox Virus S-SPV-047 and
 the DNA insertion in Homology Vector 779-94.31.
 5 Diagram showing the orientation of DNA fragments
 assembled in plasmid 779-94.31. The origin of each
 fragment is indicated in the table. The sequences
 located at each of the junctions between fragments
 is also shown. Figures 12A-12C show the sequences
 10 located at Junction A (S:), , C , D , and E
 between fragments and the sequences located at the
 junctions. The restriction sites used to generate
 each fragment as well as synthetic linker sequences
 15 which are used to join the fragments are described
 for each junction. The location of several gene
 coding regions and regulatory elements is also
 given. The following two conventions are used:
 numbers in parentheses, (), refer to amino acids,
 and restrictions sites in brackets, [], indicate the
 20 remnants of sites which are destroyed during
 construction. The following abbreviations are used:
 swinepox virus (SPV), pseudorabies virus (PRV),
 Escherichia coli (E. coli), pox synthetic late
 promoter 2 early promoter 2 (LP2EP2), pox synthetic
 25 late promoter 1 (LP1), base pairs (BP).

Figures 13A-13D:

Detailed description of Swinepox Virus S-SPV-052 and
 the DNA insertion in Homology Vector 789-41.7.
 30 Diagram showing the orientation of DNA fragments
 assembled in plasmid 789-41.7. The origin of each
 fragment is indicated in the table. The sequences
 located at each of the junctions between fragments
 is also shown. Figures 13A-13D show the sequences
 35 located at Junction A, B, C , D , E , and F between
 fragments and the sequences located at the
 junctions. The restriction sites used to generate

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each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restrictions sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), Escherichia coli (E. coli), pox synthetic late promoter 2 early promoter 2 (LP2EP2), pox synthetic early promoter 1 late promoter 2 (EP1LP2), pox synthetic late promoter 1 (LP1), base pairs (BP).

Figures 14A-14D:

Detailed description of Swinepox Virus S-SPV-053 and the DNA insertion in Homology Vector 789-41.27. Diagram showing the orientation of DNA fragments assembled in plasmid 789-41.27. The origin of each fragment is indicated in the table. The sequences located at each of the junctions between fragments is also shown. Figures 14A-14D show the sequences located at Junction A, B, C, D, E, F, and G between fragments and the sequences located at the junctions. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restrictions sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), Escherichia coli (E. coli), pox synthetic late

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promoter 2 early promoter 2 (LP2EP2), pox synthetic
early promoter 1 late promoter 2 (EP1LP2), pox
synthetic late promoter 1 (LP1), base pairs (BP).

5 **Figures 15A-15D:**

Detailed description of Swinepox Virus S-SPV-054 and
the DNA insertion in Homology Vector 789-41.47.
Diagram showing the orientation of DNA fragments
assembled in plasmid 789-41.47. The origin of each
10 fragment is indicated in the table. The sequences
located at each of the junctions between fragments
is also shown. Figures 15A-15D show the sequences
located at Junction A, B, C (SEQ ID NO:), D, E,
F, and G between fragments and the sequences
15 located at the junctions. The restriction sites
used to generate each fragment as well as synthetic
linker sequences which are used to join the
fragments are described for each junction. The
location of several gene coding regions and
20 regulatory elements is also given. The following
two conventions are used: numbers in parentheses,
(), refer to amino acids, and restrictions sites in
brackets, [], indicate the remnants of sites which
are destroyed during construction. The following
25 abbreviations are used: swinepox virus (SPV),
pseudorabies virus (PRV), Escherichia coli (E.
coli), pox synthetic early promoter 1 late promoter
2 (EP1LP2), pox synthetic late promoter 1 (LP1),
base pairs (BP).

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Figures 16A-16E:

Detailed description of Swinepox Virus S-SPV-055 and
the DNA insertion in Homology Vector 789-41.73.
35 Diagram showing the orientation of DNA fragments
assembled in plasmid 789-41.73. The origin of each
fragment is indicated in the table. The sequences

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located at each of the junctions between fragments is also shown. Figures 16A-16E show the sequences located at Junction A, B, C, D, E, F, G, and H between fragments and the sequences located at the junctions. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restrictions sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), Escherichia coli (E. coli), pox synthetic late promoter 2 early promoter 2 (LP2EP2), pox synthetic early promoter 1 late promoter 2 (EP1LP2), pox synthetic late promoter 1 (LP1), base pairs (BP).

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DETAILED DESCRIPTION OF THE INVENTION

This invention provides a recombinant swinepox virus comprising a foreign DNA inserted into a swinepox virus genome, wherein the foreign DNA is inserted into an EcoRI site within a region corresponding to a 3.2 Kb subfragment of the HindIII K fragment which contains both a HindIII and an EcoRI site, of the swinepox virus genome and is capable of being expressed in a host cell into which the virus is introduced.

This invention provides a recombinant swinepox virus comprising a foreign DNA inserted into a swinepox virus genome, wherein the foreign DNA is inserted into a) an AccI site within a region corresponding to a HindIII to BglII subfragment of the larger HindIII M fragment and b) an EcoRI site within a region corresponding to a 3.2 Kb subfragment of the HindIII K fragment which contains both a HindIII and EcoRI site, of the swinepox virus genome and is capable of being expressed in a host cell into which the virus is introduced.

In another embodiment the open reading frame encodes a B18R gene. In another embodiment the open reading frame encodes a B4R gene. In another embodiment the open reading frame encodes swinepox homologue of the 77.2 kD protein gene. In another embodiment the open reading frame encodes swinepox homologue of the T5 protein gene.

In another embodiment the foreign DNA sequence is inserted within a EcoRV restriction endonuclease site within the approximately 2 kB HindIII to BamHI subfragment of the swinepox virus genome. In another embodiment the foreign DNA sequence is inserted within a SnaBI restriction endonuclease site within the

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approximately 2.0 kB HindIII to BamHI subfragment of the swinepox virus genome.

5 In another embodiment the foreign DNA sequence is inserted within an approximately 1.2 kB BamHI to HindIII subfragment of the HindIII N fragment of the swinepox virus genome. In another embodiment the foreign DNA sequence is inserted into an open reading frame within an approximately 1.2 kB BamHI to HindIII subfragment of the
10 HindIII N fragment of the swinepox virus genome. In another embodiment the foreign DNA sequence is inserted into an open reading frame which encodes a I4L gene. In another embodiment the foreign DNA sequence is inserted within a BglII restriction endonuclease site within the
15 approximately 1.2 kB BamHI to HindIII subfragment of the swinepox virus genome.

In another embodiment the recombinant swinepox virus contains the foreign DNA sequence inserted within an
20 approximately 3.6 kB larger HindIII to BglII subfragment of the HindIII M fragment of the swinepox virus genomic DNA. In another embodiment the foreign DNA sequence is inserted into an open reading frame within an approximately 3.6 kB larger HindIII to BglII subfragment
25 of the HindIII M fragment of the swinepox virus genomic DNA. In another embodiment the open reading frame encodes a I4L gene.

30 In one embodiment the foreign DNA sequence of the recombinant swinepox virus is inserted within a non-essential Open Reading Frame (ORF) of the HindIII M fragment. Example of ORF's include, but are not limited to: I4L, I2L, 01L, and E10L.

35 In another embodiment the recombinant swinepox virus further comprises a foreign DNA sequence inserted into an open reading frame encoding swinepox virus thymidine

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kinase. In one embodiment the foreign DNA sequence is inserted into a NdeI site located within the open reading frame encoding the swinepox virus thymidine kinase.

5 For purposes of this invention, "a recombinant swinepox virus capable of replication" is a live swinepox virus which has been generated by the recombinant methods well known to those of skill in the art, e.g., the methods set forth in HOMOLOGOUS RECOMBINATION PROCEDURE FOR
10 GENERATING RECOMBINANT SPV in Materials and Methods and has not had genetic material essential for the replication of the recombinant swinepox virus deleted.

15 For purposes of this invention, "an insertion site which is not essential for replication of the swinepox virus" is a region or a region which corresponds to a specific fragment in the swinepox viral genome where a sequence of DNA is not necessary for viral replication, for example, complex protein binding sequences, sequences which code
20 for reverse transcriptase or an essential glycoprotein, DNA sequences necessary for packaging, etc.

For purposes of this invention, a "promoter" is a specific DNA sequence on the DNA molecule to which the
25 foreign RNA polymerase attaches and at which transcription of the foreign RNA is initiated.

For purposes of this invention, an "open reading frame" is a segment of DNA which contains codons that can be
30 transcribed into RNA which can be translated into an amino acid sequence and which does not contain a termination codon.

35 The invention further provides a foreign DNA sequence or foreign RNA which encodes a polypeptide. Preferably, the polypeptide is antigenic in the animal. Preferably, this antigenic polypeptide is a linear polymer of more than 10

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amino acids linked by peptide bonds which stimulates the animal to produce antibodies.

5 The S-SPV-003 swinepox virus has been deposited pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the Purposes of Patent Procedure with the Patent Culture Depository of the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A. under ATCC Accession No. VR 2335.

10

For purposes of this invention, a "polypeptide which is a detectable marker" includes the bimer, trimer and tetramer form of the polypeptide. E. coli β -galactosidase is a tetramer composed of four polypeptides or monomer sub-units.

15

Foreign DNA which code for a polypeptide include but are not limited to: feline leukemia virus surface protein, feline leukemia virus transmembrane protein, feline leukemia virus gag, feline leukemia virus transmembrane protease, feline immunodeficiency virus gag/protease, feline immunodeficiency virus envelope, feline leukemia virus gag/protease, feline leukemia virus envelope, canine parvovirus VP2, canine parvovirus VP1/2, bovine cytokine interleukin-12 protein 35, bovine cytokine interleukin-12 protein 40, Bovine Respiratory Syncytial Virus glycoprotein G, Newcastle Disease fusion, Infectious Rhinotracheitis Virus glycoprotein D, Canine Distemper Virus fusion, Canine Distemper Virus Hemagglutinin, DV HA, Bovine Viral Diarrhea Virus type 1 glycoprotein 45, Bovine Viral Diarrhea Virus type 1 glycoprotein 48, Bovine Viral Diarrhea Virus type 1 glycoprotein 53, Bovine Viral Diarrhea Virus type 2 glycoprotein 53.

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The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an antigenic polypeptide is: Swine Influenza Virus hemagglutinin, Swine Influenza Virus neurominidase, Swine Influenza Virus matrix, Swine Influenza Virus nucleoprotein, African Swine Fever Virus or *Mycoplasma hyopneumoniae*. Preferred embodiments of such virus are designated S-SPV-121, and S-SPV-122.

- 10 The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an antigenic polypeptide is: cytokine is chicken macrophage migration inhibitory factor (cMIF), chicken myelomonocytic growth factor (cMGF) or chicken interferon (cIFN). Preferred embodiments of such virus are designated S-SPV-068, and S-SPV-105.

- The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an antigenic polypeptide is: porcine reproductive and respiratory syndrome virus (PRRS) ORF2, ORF3, ORF4, ORF5, ORF6 and ORF7, pseudorabies gB, gD, gI. Preferred embodiments of such virus are designated S-SPV-076, S-SPV-079, S-SPV-090, S-SPV-084, S-SPV-091, S-SPV-092, S-SPV-093, S-SPV-094, S-SPV-095.

- The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an antigenic polypeptide is: Infectious bovine rhinotracheitis virus glycoprotein B, glycoprotein D and glycoprotein I, bovine respiratory syncytial virus attachment protein (BRSV G), bovine respiratory syncytial virus fusion protein (BRSV F), bovine respiratory syncytial virus nucleocapsid protein (BRSV N). Preferred embodiments of such virus are designated S-SPV-109, S-

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SPV-110, S-SPV-111, S-SPV-113, S-SPV-115, S-SPV-119, S-SPV-112.

5 The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an antigenic polypeptide is: bovine viral diarrhea virus (BVDV) glycoprotein 48 or glycoprotein 53. A Preferred embodiment of such a virus is designated S-SPV-099.

10 The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an antigenic polypeptide is: feline immunodeficiency virus gag/protease and envelope, feline leukemia virus gag/protease and envelope. Preferred embodiments of such
15 viruses are designated: S-SPV-106, S-SPV-089, S-SPV-100, S-SPV-107, S-SPV-108.

The present invention further provides a recombinant swinepox virus in which the foreign DNA encodes an
20 antigenic polypeptide is: canine parvovirus VP2 and VP1/2. Preferred embodiments of such viruses are designated: S-SPV-114, S-SPV-116, S-SPV-117, S-SPV-118.

The present invention provides a recombinant swinepox
25 virus comprising a foreign DNA inserted into the swinepox virus genomic DNA, wherein the one or more foreign DNAs are inserted within each of the HindIII K fragment of the swinepox virus genomic DNA and within the HindIII M
30 fragment of the swinepox virus genomic DNA and is capable of being expressed in a swinepox virus infected host cell. Preferred embodiments of such viruses are designated: S-SPV-127, S-SPV-128, S-SPV-131, and S-SPV-132.

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The present invention provides a recombinant swinepox virus comprising a foreign DNA inserted into the swinepox virus genomic DNA, wherein the one or more foreign DNAs which encode a fusion protein are inserted within each of
5 the HindIII K fragment of the swinepox virus genomic DNA and within the HindIII M fragment of the swinepox virus genomic DNA and is capable of being expressed in a swinepox virus infected host cell. Preferred embodiments of such viruses are designated: S-SPV-130.

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The invention further provides a recombinant swinepox virus capable of replication which contains foreign DNA encoding an antigenic polypeptide which is or is from
15 pseudorabies virus (PRV) g50 (gD), pseudorabies virus (PRV) gII (gB), Pseudorabies virus (PRV) gIII (gC), pseudorabies virus (PRV) glycoprotein H, pseudorabies virus (PRV) glycoprotein E, Transmissible gastroenteritis (TGE) glycoprotein 195, Transmissible gastroenteritis (TGE) matrix protein, swine rotavirus glycoprotein 38,
20 swine parvovirus capsid protein, *Serpulina hyodysenteriae* protective antigen, Bovine Viral Diarrhea (BVD) glycoprotein 55, Newcastle Disease Virus (NDV) hemagglutinin-neuraminidase, swine flu hemagglutinin or swine flu neuraminidase. Preferably, the antigenic
25 polypeptide is Pseudorabies Virus (PRV) g50 (gD). Preferably, the antigenic protein is Newcastle Disease Virus (NDV) hemagglutinin-neuraminidase.

The invention further provides a recombinant swinepox
30 virus capable of replication which contains foreign DNA encoding an antigenic polypeptide which is or is from *Serpulina hyodysenteriae*, Foot and Mouth Disease Virus, Hog Cholera Virus, African Swine Fever Virus or *Mycoplasma hyopneumoniae*.

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The invention further provides for a recombinant swinepox virus capable of replication which contains foreign DNA encoding RNA encoding the antigenic polypeptide Newcastle Disease Virus (NDV) hemagglutinin-neuraminidase further comprising foreign DNA encoding a polypeptide which is a detectable marker.

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from infectious bovine rhinotracheitis virus and is capable of being expressed in a host infected by the recombinant swinepox virus. Examples of such antigenic polypeptide are infectious bovine rhinotracheitis virus glycoprotein E and glycoprotein G.

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from infectious laryngotracheitis virus and is capable of being expressed in a host infected by the recombinant swinepox virus. Examples of such antigenic polypeptide are infectious laryngotracheitis virus glycoprotein G and glycoprotein I.

In one embodiment of the recombinant swinepox virus the foreign DNA sequence encodes a cytokine. In another embodiment the cytokine is chicken myelomonocytic growth factor (cMGF) or chicken interferon (cIFN). Cytokines include, but are not limited to: transforming growth factor beta, epidermal growth factor family, fibroblast growth factors, hepatocyte growth factor, insulin-like growth factor, vascular endothelial growth factor,

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interleukin 1, IL-1 receptor antagonist, interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, IL-6 soluble receptor, interleukin-7, interleukin-8, interleukin-9, interleukin-10, interleukin-11, interleukin-12, interleukin-13, angiogenin, chemokines, colony stimulating factors, granulocyte-macrophage colony stimulating factors, erythropoietin, interferon, interferon gamma, Stem cell factor (or known as mast cell growth factor, or c-kit ligand protein), leukemia inhibitory factor, oncostatin M, pleiotrophin, secretory leukocyte protease inhibitor, stem cell factor, tumor necrosis factors, and soluble TNF receptors. These cytokines are from humans, bovine, equine, feline, canine, porcine or avian.

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from a human pathogen and is capable of being expressed in a host infected by the recombinant swinepox virus.

Recombinant SPV expressing cytokines is used to enhance the immune response either alone or when combined with vaccines containing cytokines or antigen genes of disease causing microorganisms.

Antigenic polypeptide of a human pathogen which are derived from human herpesvirus include, but are not limited to: hepatitis B virus and hepatitis C virus hepatitis B virus surface and core antigens, hepatitis C virus, human immunodeficiency virus, herpes simplex virus-1, herpes simplex virus-2, human cytomegalovirus, Epstein-Barr virus, Varicella-Zoster virus, human herpesvirus-6, human herpesvirus-7, human influenza, measles virus, hantaan virus, pneumonia virus,

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rhinovirus, poliovirus, human respiratory syncytial virus, retrovirus, human T-cell leukemia virus, rabies virus, mumps virus, malaria (*Plasmodium falciparum*), *Bordetella pertussis*, Diphtheria, *Rickettsia prowazekii*,
5 *Borrelia berfordorferi*, Tetanus toxoid, malignant tumor antigens.

In one embodiment of the invention, a recombinant swinepox virus contains the foreign DNA sequence encoding
10 hepatitis B virus core protein.

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox
15 genome, wherein the foreign DNA sequence encodes a cytokine capable of stimulating an immune in a host infected by the recombinant swinepox virus and is capable of being expressed in the host infected.

20

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an
25 antigenic polypeptide derived from an equine pathogen and is capable of being expressed in a host infected by the recombinant swinepox virus.

The antigenic polypeptide of an equine pathogen can
30 derived from equine influenza virus, or equine herpesvirus. In one embodiment the antigenic polypeptide is equine influenza neuraminidase or hemagglutinin. Examples of such antigenic polypeptide are equine influenza virus type A/Alaska 91 neuraminidase, equine
35 influenza virus type A/Prague 56 neuraminidase, equine influenza virus type A/Miami 63 neuraminidase, equine influenza virus type A/Kentucky 81 neuraminidase, equine

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influenza virus type A/Kentucky 92 neuraminidase equine
herpesvirus type 1 glycoprotein B, equine herpesvirus
type 1 glycoprotein D, *Streptococcus equi*, equine
infectious anemia virus, equine encephalitis virus,
5 equine rhinovirus and equine rotavirus.

The present invention further provides an antigenic
polypeptide which includes, but is not limited to: hog
cholera virus gE1, hog cholera virus gE2, swine influenza
10 virus hemagglutinin, neurominidase, matrix and
nucleoprotein, pseudorabies virus gB, gC and gD, and PRRS
virus ORF7.

The present invention further provides a recombinant
15 swinepox virus which comprises a foreign DNA sequence
inserted into a non-essential site of the swinepox
genome, wherein the foreign DNA sequence encodes an
antigenic polypeptide derived from bovine respiratory
syncytial virus or bovine parainfluenza virus, and is
20 capable of being expressed in a host infected by the
recombinant swinepox virus.

For example, the antigenic polypeptide of derived from
infectious bovine rhinotracheitis virus gE, bovine
25 respiratory syncytial virus equine pathogen can derived
from equine influenza virus is bovine respiratory
syncytial virus attachment protein (BRSV G), bovine
respiratory syncytial virus fusion protein (BRSV F),
bovine respiratory syncytial virus nucleocapsid protein
30 (BRSV N), bovine parainfluenza virus type 3 fusion
protein, and the bovine parainfluenza virus type 3
hemagglutinin neuraminidase.

The present invention further provides a recombinant
35 swinepox virus which comprises a foreign DNA sequence
inserted into a non-essential site of the swinepox
genome, wherein the foreign DNA sequence encodes bovine

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viral diarrhea virus (BVDV) glycoprotein 48 or glycoprotein 53, and wherein the foreign DNA sequence is capable of being expressed in a host infected by the recombinant swinepox virus.

5

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from infectious bursal disease virus and wherein the foreign DNA sequence is capable of being expressed in a host infected by the recombinant swinepox virus. Examples of such antigenic polypeptide are infectious bursal disease virus polyprotein and VP2.

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The present invention further provides a recombinant swinepox virus in which the foreign DNA sequence encodes an antigenic polypeptide which includes, but is not limited to: MDV gA, MDV gB, MDV gD, NDV HN, NDV F, ILT gB, ILT gI, ILT gD, IBDV VP2, IBDV VP3, IBDV VP4, IBDV polyprotein, IBV spike, IBV matrix, avian encephalomyelitis virus, avian reovirus, avian paramyxovirus, avian influenza virus, avian adenovirus, fowl pox virus, avian coronavirus, avian rotavirus, chick anemia virus, *Salmonella* spp., *E. coli*, *Pasteurella* spp., *Bordetella* spp., *Eimeria* spp., *Histomonas* spp., *Trichomonas* spp., Poultry nematodes, cestodes, trematodes, poultry mites/lice, and poultry protozoa.

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The invention further provides that the inserted foreign DNA sequence is under the control of a promoter. In one embodiment there is a swinepox viral promoter. In another embodiment the foreign DNA sequence is under control of an endogenous upstream poxvirus promoter. In another embodiment the foreign DNA sequence is under control of a heterologous upstream promoter.

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For purposes of this invention, promoters include but is not limited to: synthetic pox viral promoter, pox synthetic late promoter 1, pox synthetic late promoter 2 early promoter 2, pox O1L promoter, pox I4L promoter, pox I3L promoter, pox I2L promoter, pox I1L promoter, pox E10R promoter, PRV gX, HSV-1 alpha 4, HCMV immediate early, MDV gA, MDV gB, MDV gD, ILT gB, BHV-1.1 VP8 and ILT gD and internal ribosomal entry site promoter. Alternate promoters are generated by methods well known to those of skill in the art, for example, as set forth in the STRATEGY FOR THE CONSTRUCTION OF SYNTHETIC POX VIRAL PROMOTERS in Materials and Methods.

The invention provides for a homology vector for producing a recombinant swinepox virus by inserting foreign DNA into the genomic DNA of a swinepox virus. The homology vector comprises a double-stranded DNA molecule consisting essentially of a double-stranded foreign DNA sequence or (RNA) which does not naturally occur in an animal into which the recombinant swinepox virus is introduced, with at one end of the foreign DNA, double-stranded swinepox viral DNA homologous to genomic DNA located at one side of a site on the genomic DNA which is not essential for replication of the swinepox virus, and at the other end of the foreign DNA, double-stranded swinepox viral DNA homologous to genomic DNA located at the other side of the same site on the genomic DNA. Preferably, the RNA encodes a polypeptide.

In another embodiment of the present invention, the double-stranded swinepox viral DNA of the homology vectors described above is homologous to genomic DNA present within the HindIII M fragment. In another embodiment the double-stranded swinepox viral DNA of the homology vectors described above is homologous to genomic DNA present within an approximately 2 Kb HindIII to BglII sub-fragment. In a preferred embodiment the double-

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stranded swinepox viral DNA is homologous to genomic DNA present within the *Bgl*III site located in this *Hind*III to *Bgl*III subfragment.

- 5 In another embodiment the double-stranded swinepox viral DNA is homologous to genomic DNA present within the open reading frame contained in the larger *Hind*III to *Bgl*III subfragment. Preferably, the double-stranded swinepox viral DNA is homologous to genomic DNA present within the
10 *Acc*I restriction endonuclease site located in the larger *Hind*III to *Bgl*III subfragment.

In one embodiment, the polypeptide is a detectable marker. Preferably, the polypeptide which is a
15 detectable marker is *E. coli* β -galactosidase.

In one embodiment, the foreign DNA which codes for a polypeptide include but are not limited to: feline leukemia virus surface protein, feline leukemia virus
20 transmembrane protein, feline leukemia virus gag, feline leukemia virus transmembrane protease, feline immunodeficiency virus gag/protease, feline immunodeficiency virus envelope, feline leukemia virus gag/protease, feline leukemia virus envelope, canine
25 parvovirus VP2, canine parvovirus VP1/2, bovine cytokine interleukin-12 protein 35, bovine cytokine interleukin-12 protein 40, Bovine Respiratory Syncytial Virus glycoprotein G, Newcastle Disease fusion, Infectious Rhinotracheitis Virus glycoprotein D, Canine Distemper
30 Virus fusion, Canine Distemper Virus Hemagglutinin, DV HA, Bovine Viral Diarrhea Virus type 1 glycoprotein 45, Bovine Viral Diarrhea Virus type 1 glycoprotein 48, Bovine Viral Diarrhea Virus type 1 glycoprotein 53, Bovine Viral Diarrhea Virus type 2 glycoprotein 53.

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- Other polypeptides include: pseudorabies virus (PRV) g50 (gD), pseudorabies virus (PRV) gII (gB), Pseudorabies virus (PRV) gIII (gC), Pseudorabies virus (PRV) glycoprotein H, Transmissible gastroenteritis (TGE) glycoprotein 195, Transmissible gastroenteritis (TGE) matrix protein, swine rotavirus glycoprotein 38, swine parvovirus capsid protein, *Serpulina hyodysenteriae* protective antigen, Bovine Viral Diarrhea (BVD) glycoprotein 53 and g48, Newcastle Disease Virus (NDV) hemagglutinin-neuraminidase, swine flu hemagglutinin or swine flu neuraminidase. Preferably, the antigenic polypeptide is or is from *Serpulina hyodysenteriae*, Foot and Mouth Disease Virus, Hog Cholera Virus gE1 and gE2, Swine Influenza Virus, African Swine Fever Virus or *Mycoplasma hyopneumoniae*, swine influenza virus hemagglutinin, neuraminidase and matrix and nucleoprotein, PRRS virus ORF7, and hepatitis B virus core protein.
- 20 In one embodiment, the polypeptide is antigenic in the animal.

In an embodiment of the present invention, the double stranded foreign DNA sequence in the homology vector encodes an antigenic polypeptide derived from a human pathogen.

For example, the antigenic polypeptide of a human pathogen is derived from human herpesvirus, herpes simplex virus-1, herpes simplex virus-2, human cytomegalovirus, Epstein-Barr virus, Varicell-Zoster virus, human herpesvirus-6, human herpesvirus-7, human influenza, human immunodeficiency virus, rabies virus, measles virus, hepatitis B virus and hepatitis C virus.

35 Furthermore, the antigenic polypeptide of a human pathogen may be associated with malaria or malignant

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tumor from the group consisting of *Plasmodium falciparum*, *Bordetella pertussis*, and malignant tumor.

5 In an embodiment of the present invention, the double stranded foreign DNA sequence in the homology vector encodes a cytokine capable of stimulating human immune response. In one embodiment the cytokine is a chicken myelomonocytic growth factor (cMGF) or chicken interferon (cIFN). For example, the cytokine can be, but not
10 limited to, interleukin-2, interleukin-6, interleukin-12, interferons, granulocyte-macrophage colony stimulating factors, and interleukin receptors.

15 In an embodiment of the present invention, the double stranded foreign DNA sequence in the homology vector encodes an antigenic polypeptide derived from an equine pathogen.

20 The antigenic polypeptide of an equine pathogen can derived from equine influenza virus or equine herpesvirus. Examples of such antigenic polypeptide are equine influenza virus type A/Alaska 91 neuraminidase, equine influenza virus type A/Prague 56 neuraminidase, equine influenza virus type A/Miami 63 neuraminidase,
25 equine influenza virus type A/Kentucky 81 neuraminidase equine herpesvirus type 1 glycoprotein B, and equine herpesvirus type 1 glycoprotein D.

30 In an embodiment of the present invention, the double stranded foreign DNA sequence of the homology vector encodes an antigenic polypeptide derived from bovine respiratory syncytial virus or bovine parainfluenza virus.

35 For example, the antigenic polypeptide is derived from infectious bovine rhinotracheitis gE, bovine respiratory syncytial virus attachment protein (BRSV G), bovine

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respiratory syncytial virus fusion protein (BRSV F),
bovine respiratory syncytial virus nucleocapsid protein
(BRSV N), bovine parainfluenza virus type 3 fusion
protein, and the bovine parainfluenza virus type 3
5 hemagglutinin neuraminidase.

In an embodiment of the present invention, the double
stranded foreign DNA sequence of the homology vector
encodes an antigenic polypeptide derived from infectious
10 bursal disease virus. Examples of such antigenic
polypeptide are infectious bursal disease virus
polyprotein and infectious bursal disease virus VP2, VP3,
or VP4.

15 For purposes of this invention, a "homology vector" is a
plasmid constructed to insert foreign DNA in a specific
site on the genome of a swinepox virus.

In one embodiment of the invention, the double-stranded
20 swinepox viral DNA of the homology vectors described
above is homologous to genomic DNA present within the
open reading frame encoding swinepox thymidine kinase.
Preferably, the double-stranded swinepox viral DNA is
homologous to genomic DNA present within the NdeI
25 restriction endonuclease site located in the open reading
frame encoding swinepox thymidine kinase.

The invention further provides a homology vectors
described above, the foreign DNA sequence of which is
30 under control of a promoter located upstream of the
foreign DNA sequence. The promoter can be an endogenous
swinepox viral promoter or an exogenous promoter.
Promoters include, but are not limited to: synthetic pox
viral promoter, pox synthetic late promoter 1, pox
35 synthetic late promoter 2 early promoter 2, pox O1L
promoter, pox I4L promoter, pox I3L promoter, pox I2L
promoter, pox I1L promoter, pox E10R promoter, PRV gX,

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HSV-1 alpha 4, HCMV immediate early, BHV-1.1 VP8, infectious laryngotracheitis virus glycoprotein B, infectious laryngotracheitis virus gD, marek's disease virus glycoprotein A, marek's disease virus glycoprotein B, and marek's disease virus glycoprotein D.

The invention further provides a vaccine which comprises an effective immunizing amount of a recombinant swinepox virus of the present invention and a suitable carrier.

Suitable carriers for the swinepox virus are well known in the art and include proteins, sugars, etc. One example of such a suitable carrier is a physiologically balanced culture medium containing one or more stabilizing agents such as stabilized, hydrolyzed proteins, lactose, etc.

For purposes of this invention, an "effective immunizing amount" of the recombinant swinepox virus of the present invention is within the range of 10^3 to 10^9 PFU/dose.

The present invention also provides a method of immunizing an animal, wherein the animal is a human, swine, bovine, equine, caprine or ovine. For purposes of this invention, this includes immunizing the animal against the virus or viruses which cause the disease or diseases pseudorabies, transmissible gastroenteritis, swine rotavirus, swine parvovirus, *Serpulina hyodysenteriae*, bovine viral diarrhea, Newcastle disease, swine influenza, PRRS, bovine respiratory syncytial virus, bovine parainfluenza virus type 3, foot and mouth disease, hog cholera, African swine fever or *Mycoplasma hyopneumoniae*. For purposes of this invention, the method of immunizing also includes immunizing the animal against human pathogens, bovine pathogens, equine

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pathogens, avian pathogens described in the preceding part of this section.

5 The method comprises administering to the animal an effective immunizing dose of the vaccine of the present invention. The vaccine may be administered by any of the methods well known to those skilled in the art, for example, by intramuscular, subcutaneous, intraperitoneal or intravenous injection. Alternatively, the vaccine may
10 be administered intranasally or orally.

The present invention also provides a method for testing a swine to determine whether the swine has been vaccinated with the vaccine of the present invention,
15 particularly the embodiment which contains the recombinant swinepox virus S-SPV-008 (ATCC Accession No. VR 2339), or is infected with a naturally-occurring, wild-type pseudorabies virus. This method comprises obtaining from the swine to be tested a sample of a
20 suitable body fluid, detecting in the sample the presence of antibodies to pseudorabies virus, the absence of such antibodies indicating that the swine has been neither vaccinated nor infected, and for the swine in which antibodies to pseudorabies virus are present, detecting
25 in the sample the absence of antibodies to pseudorabies virus antigens which are normally present in the body fluid of a swine infected by the naturally-occurring pseudorabies virus but which are not present in a vaccinated swine indicating that the swine was vaccinated
30 and is not infected.

The present invention provides a recombinant SPV which when inserted with a foreign DNA sequence or gene may be employed as a diagnostic assay. In one embodiment FIV
35 env and gag genes and *D. immitis* p39 and 22kd are employed in a diagnostic assay to detect feline

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immunodeficiency caused by FIV and to detect heartworm caused by *D. immitis*, respectively.

5 The present invention also provides a host cell infected with a recombinant swinepox virus capable of replication. In one embodiment, the host cell is a mammalian cell. Preferably, the mammalian cell is a Vero cell. Preferably, the mammalian cell is an ESK-4 cell, PK-15 cell or EMSK cell.

10

For purposes of this invention a "host cell" is a cell used to propagate a vector and its insert. Infecting the cells was accomplished by methods well known to those of skill in the art, for example, as set forth in INFECTION
15 - TRANSFECTION PROCEDURE in Material and Methods.

20

Methods for constructing, selecting and purifying recombinant swinepox viruses described above are detailed below in Materials and Methods.

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EXPERIMENTAL DETAILSMaterials and Methods

- 5 **PREPARATION OF SWINEPOX VIRUS STOCK SAMPLES.** Swinepox virus (SPV) samples were prepared by infecting embryonic swine kidney (EMSK) cells, ESK-4 cells, PK-15 cells or Vero cells at a multiplicity of infection of 0.01 PFU/cell in a 1:1 mixture of Iscove's Modified Dulbecco's
10 Medium (IMDM) and RPMI 1640 medium containing 2 mM glutamine, 100 units/ml penicillin, 100 units/ml streptomycin (these components were obtained from Sigma or equivalent supplier, and hereafter are referred to as EMSK negative medium). Prior to infection, the cell
15 monolayers were washed once with EMSK negative medium to remove traces of fetal bovine serum. The SPV contained in the initial inoculum (0.5 ml for 10 cm plate; 10 ml for T175 cm flask) was then allowed to absorb onto the cell monolayer for two hours, being redistributed every half
20 hour. After this period, the original inoculum was brought up to the recommended volume with the addition of complete EMSK medium (EMSK negative medium plus 5% fetal bovine serum). The plates were incubated at 37°C in 5% CO₂ until cytopathic effect was complete. The medium and
25 cells were harvested and frozen in a 50 ml conical screw cap tube at -70°C. Upon thawing at 37°C, the virus stock was aliquoted into 1.0 ml vials and refrozen at -70°C. The titers were usually about 10⁶ PFU/ml.
- 30 **PREPARATION OF SPV DNA.** For swinepox virus DNA isolation, a confluent monolayer of EMSK cells in a T175 cm² flask was infected at a multiplicity of 0.1 and incubated 4-6 days until the cells were showing 100% cytopathic effect. The infected cells were then harvested by scraping the
35 cells into the medium and centrifuging at 3000 rpm for 5 minutes in a clinical centrifuge. The medium was decanted, and the cell pellet was gently resuspended in

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1.0 ml Phosphate Buffer Saline (PBS: 1.5g Na_2HPO_4 , 0.2g KH_2PO_4 , 0.8g NaCl and 0.2g KCl per liter H_2O) (per T175) and subjected to two successive freeze-thaws (-70°C to 37°C). Upon the last thaw, the cells (on ice) were
5 sonicated two times for 30 seconds each with 45 seconds cooling time in between. Cellular debris was then removed by centrifuging (Sorvall RC-5B superspeed centrifuge) at 3000 rpm for 5 minutes in a HB4 rotor at 4°C . SPV virions, present in the supernatant, were then pelleted
10 by centrifugation at 15,000 rpm for 20 minutes at 4°C in a SS34 rotor (Sorvall) and resuspended in 10 mM Tris (pH 7.5). This fraction was then layered onto a 36% sucrose gradient (w/v in 10 mM tris pH 7.5) and centrifuged (Beckman L8-70M Ultracentrifuge) at 18,000 rpm for 60
15 minutes in a SW41 rotor (Beckman) at 4°C . The virion pellet was resuspended in 1.0 ml of 10 mM tris pH 7.5 and sonicated on ice for 30 seconds. This fraction was layered onto a 20% to 50% continuous sucrose gradient and centrifuged 16,000 rpm for 60 minutes in a SW41 rotor at
20 4°C . The SPV virion band located about three quarters down the gradient was harvested, diluted with 20% sucrose and pelleted by centrifugation at 18,000 rpm for 60 minutes in a SW41 rotor at 4°C . The resultant pellet was then washed once with 10 mM Tris pH 7.5 to remove traces
25 of sucrose and finally resuspended in 10 mM Tris pH 7.5. SPV DNA was then extracted from the purified virions by lysis (4 hours at 60°C) induced by the addition of EDTA, SDS, and proteinase K to final concentrations of 20 mM, 0.5% and 0.5 mg/ml, respectively. After digestion, three
30 phenol:chloroform (1:1) extractions were conducted and the sample precipitated by the addition of two volumes of absolute ethanol and incubation at -20°C for 30 minutes. The sample was then centrifuged in an Eppendorf minifuge for 5 minutes at full speed. The supernatant was
35 decanted, and the pellet air dried and rehydrated in 0.01 M Tris pH 7.5, 1 mM EDTA at 4°C .

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PREPARATION OF INFECTED CELL LYSATES. For cell lysate preparation, serum free medium was used. A confluent monolayer of cells (EMSK, ESK-4, PK-15 or Vero for SPV or VERO for PRV) in a 25 cm² flask or a 60 mm petri dish was infected with 100 μ l of virus sample. After cytopathic effect was complete, the medium and cells were harvested and the cells were pelleted at 3000 rpm for 5 minutes in a clinical centrifuge. The cell pellet was resuspended in 250 μ l of disruption buffer (2% sodium dodecyl sulfate, 2% β -mercapto-ethanol). The samples were sonicated for 30 seconds on ice and stored at -20°C.

WESTERN BLOTTING PROCEDURE. Samples of lysates and protein standards were run on a polyacrylamide gel according to the procedure of Laemmli (1970). After gel electrophoresis the proteins were transferred and processed according to Sambrook et al. (1982). The primary antibody was a swine anti-PRV serum (Shope strain; lot370, PDV8201, NVSL, Ames, IA) diluted 1:100 with 5% non-fat dry milk in Tris-sodium chloride, and sodium Azide (TSA: 6.61g Tris-HCl, 0.97g Tris-base, 9.0g NaCl and 2.0g Sodium Azide per liter H₂O). The secondary antibody was a goat anti-swine alkaline phosphatase conjugate diluted 1:1000 with TSA.

MOLECULAR BIOLOGICAL TECHNIQUES. Techniques for the manipulation of bacteria and DNA, including such procedures as digestion with restriction endonucleases, gel electrophoresis, extraction of DNA from gels, ligation, phosphorylation with kinase, treatment with phosphatase, growth of bacterial cultures, transformation of bacteria with DNA, and other molecular biological methods are described by Maniatis et al. (1982) and Sambrook et al. (1989). Except as noted, these were used with minor variation.

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DNA SEQUENCING. Sequencing was performed using the USB Sequenase Kit and ³⁵S-dATP (NEN). Reactions using both the dGTP mixes and the dITP mixes were performed to clarify areas of compression. Alternatively, compressed areas were resolved on formamide gels. Templates were double-stranded plasmid subclones or single stranded M13 subclones, and primers were either made to the vector just outside the insert to be sequenced, or to previously obtained sequence. Sequence obtained was assembled and compared using Dnastar software. Manipulation and comparison of sequences obtained was performed with Superclone™ and Supersee™ programs from Coral Software.

CLONING WITH THE POLYMERASE CHAIN REACTION. The polymerase chain reaction (PCR) was used to introduce restriction sites convenient for the manipulation of various DNAs. The procedures used are described by Innis, et al. (1990). In general, amplified fragments were less than 500 base pairs in size and critical regions of amplified fragments were confirmed by DNA sequencing. The primers used in each case are detailed in the descriptions of the construction of homology vectors below.

HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. This method relies upon the homologous recombination between the swinepox virus DNA and the plasmid homology vector DNA which occurs in the tissue culture cells containing both swinepox virus DNA and transfected plasmid homology vector. For homologous recombination to occur, the monolayers of EMSK cells are infected with S-SPV-001 (Kasza SPV strain, 17) at a multiplicity of infection of 0.01 PFU/cell to introduce replicating SPV (i.e. DNA synthesis) into the cells. The plasmid homology vector DNA is then transfected into these cells according to the INFECTION - TRANSFECTION

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PROCEDURE. The construction of homology vectors used in this procedure is described below

INFECTION - TRANSFECTION PROCEDURE. 6 cm plates of EMSK cells (about 80% confluent) were infected with S-SPV-001 at a multiplicity of infection of 0.01 PFU/cell in EMSK negative medium and incubated at 37°C in a humidified 5% CO₂ environment for 5 hours. The transfection procedure used is essentially that recommended for Lipofectin™ Reagent (BRL). Briefly, for each 6 cm plate, 15 µg of plasmid DNA was diluted up to 100 µl with H₂O. Separately, 50 micrograms of Lipofectin Reagent was diluted to 100 µl with H₂O. The 100 µl of diluted Lipofectin Reagent was then added dropwise to the diluted plasmid DNA contained in a polystyrene 5 ml snap cap tube and mixed gently. The mixture was then incubated for 15-20 minutes at room temperature. During this time, the virus inoculum was removed from the 6 cm plates and the cell monolayers washed once with EMSK negative medium. Three ml of EMSK negative medium was then added to the plasmid DNA/lipofectin mixture and the contents pipetted onto the cell monolayer. The cells were incubated overnight (about 16 hours) at 37°C in a humidified 5% CO₂ environment. The next day the 3 ml of EMSK negative medium was removed and replaced with 5 ml EMSK complete medium. The cells were incubated at 37°C in 5% CO₂ for 3-7 days until cytopathic effect from the virus was 80-100%. Virus was harvested as described above for the preparation of virus stocks. This stock was referred to as a transfection stock and was subsequently screened for recombinant virus by the BLUOGAL SCREEN FOR RECOMBINANT SWINEPOX VIRUS OR CPRG SCREEN FOR RECOMBINANT SWINEPOX VIRUS.

SCREEN FOR RECOMBINANT SPV EXPRESSING β-galactosidase (BLUOGAL AND CPRG ASSAYS). When the *E. coli* β-galactosidase (lacZ) marker gene was incorporated into a

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recombinant virus the plaques containing the recombinants were visualized by one of two simple methods. In the first method, the chemical Bluogal™ (Bethesda Research Labs) was incorporated (200 µg/ml) into the agarose overlay during the plaque assay, and plaques expressing active β-galactosidase turned blue. The blue plaques were then picked onto fresh cells (EMSK) and purified by further blue plaque isolation. In the second method, CPRG (Boehringer Mannheim) was incorporated (400 µg/ml) into the agarose overlay during the plaque assay, and plaques expressing active β-galactosidase turned red. The red plaques were then picked onto fresh cells (EMSK) and purified by further red plaque isolation. In both cases viruses were typically purified with three rounds of plaque purification.

SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV USING BLACK PLAQUE ASSAYS. To analyze expression of foreign antigens expressed by recombinant swinepox viruses, monolayers of EMSK cells were infected with recombinant SPV, overlaid with nutrient agarose media and incubated for 6-7 days at 37°C for plaque development to occur. The agarose overlay was then removed from the dish, the cells fixed with 100% methanol for 10 minutes at room temperature and the cells air dried. Fixation of the cells results in cytoplasmic antigen as well as surface antigen detection whereas specific surface antigen expression can be detected using non-fixed cells. The primary antibody was then diluted to the appropriate dilution with PBS and incubated on the cell monolayer for 2 hours at room temperature. To detect PRV g50 (gD) expression from S-SPV-008, swine anti-PRV serum (Shope strain; lot370, PDV8201, NVSL, Ames, IA) was used (diluted 1:100). To detect NDV HN expression from S-SPV-009, a rabbit antiserum specific for the HN protein (rabbit anti-NDV#2) was used (diluted 1:1000). Unbound antibody was then removed by washing the cells three

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times with PBS at room temperature. The secondary antibody, either a goat anti-swine (PRV g50 (gD); S-SPV-008) or goat anti-rabbit (NDV HN; S-SPV-009), horseradish peroxidase conjugate was diluted 1:250 with PBS and
5 incubated with the cells for 2 hours at room temperature. Unbound secondary antibody was then removed by washing the cells three times with PBS at room temperature. The cells were then incubated 15-30 minutes at room
10 temperature with freshly prepared substrate solution (100 μ g/ml 4-chloro-1-naphthol, 0.003% H_2O_2 in PBS). Plaques expressing the correct antigen stain black.

PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS. Viral glycoproteins are purified using
15 antibody affinity columns. To produce monoclonal antibodies, 8 to 10 week old BALB/c female mice are vaccinated intraperitoneally seven times at two to four week intervals with 10^7 PFU of S-SPV-009, -014, -016, -
017, -018, or -019. Three weeks after the last
20 vaccination, mice are injected intraperitoneally with 40 mg of the corresponding viral glycoprotein. Spleens are removed from the mice three days after the last antigen dose.

25 Splenocytes are fused with mouse NS1/Ag4 plasmacytoma cells by the procedure modified from Oi and Herzenberg, (41). Splenocytes and plasmacytoma cells are pelleted together by centrifugation at 300 x g for 10 minutes. One ml of a 50% solution of polyethylene glycol (m.w.
30 1300-1600) is added to the cell pellet with stirring over one minute. Dulbecco's modified Eagles's medium (5ml) is added to the cells over three minutes. Cells are pelleted by centrifugation at 300 x g for 10 minutes and resuspended in medium with 10% fetal bovine serum and
35 containing 100 mM hypoxanthine, 0.4 mM aminopterin and 16 mM thymidine (HAT). Cells (100 ml) are added to the wells of eight to ten 96-well tissue culture plates

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containing 100 ml of normal spleen feeder layer cells and incubated at 37°C. Cells are fed with fresh HAT medium every three to four days.

- 5 Hybridoma culture supernatants are tested by the ELISA ASSAY in 96-well microtiter plates coated with 100 ng of viral glycoprotein. Supernatants from reactive hybridomas are further analyzed by black-plaque assay and by Western Blot. Selected hybridomas are cloned twice by
10 limiting dilution. Ascetic fluid is produced by intraperitoneal injection of 5×10^6 hybridoma cells into pristane-treated BALB/c mice.

- Cell lysates from S-SPV-009, -014, -016, -017, -018, or
15 -019 are obtained as described in PREPARATION OF INFECTED CELL LYSATES. The glycoprotein-containing cell lysates (100 mls) are passed through a 2-ml agarose affinity resin to which 20 mg of glycoprotein monoclonal antibody has been immobilized according to manufacturer's
20 instructions (AFC Medium, New Brunswick Scientific, Edison, N.J.). The column is washed with 100 ml of 0.1% Nonidet P-40 in phosphate-buffered saline (PBS) to remove nonspecifically bound material. Bound glycoprotein is eluted with 100 mM carbonate buffer, pH 10.6 (40). Pre-
25 and posteluted fractions are monitored for purity by reactivity to the SPV monoclonal antibodies in an ELISA system.

- ELISA ASSAY. A standard enzyme-linked immunosorbent
30 assay (ELISA) protocol is used to determine the immune status of cattle following vaccination and challenge.

- A glycoprotein antigen solution (100 ml at ng/ml in PBS) is allowed to absorb to the wells of microtiter dishes
35 for 18 hours at 4°C. The coated wells are rinsed one time with PBS. Wells are blocked by adding 250 ml of PBS containing 1% BSA (Sigma) and incubating 1 hour at 37°C.

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The blocked wells are rinsed one time with PBS containing 0.02% Tween 20. 50 ml of test serum (previously diluted 1:2 in PBS containing 1% BSA) are added to the wells and incubated 1 hour at 37°C. The antiserum is removed and the wells are washed 3 times with PBS containing 0.02% Tween 20. 50 ml of a solution containing anti-bovine IgG coupled to horseradish peroxidase (diluted 1:500 in PBS containing 1% BSA, Kirkegaard and Perry Laboratories, Inc.) is added to visualize the wells containing antibody against the specific antigen. The solution is incubated 1 hour at 37°C, then removed and the wells are washed 3 times with PBS containing 0.02% Tween 20. 100 ml of substrate solution (ATBS, Kirkegaard and Perry Laboratories, Inc.) are added to each well and color is allowed to develop for 15 minutes. The reaction is terminated by addition of 0.1M oxalic acid. The color is read at absorbance 410nm on an automatic plate reader.

STRATEGY FOR THE CONSTRUCTION OF SYNTHETIC POX VIRAL PROMOTERS. For recombinant swinepox vectors synthetic pox promoters offer several advantages including the ability to control the strength and timing of foreign gene expression. Three promoter cassettes LP1, EP1 and LP2 based on promoters that have been defined in the vaccinia virus (1, 7 and 8) were designed. Each cassette was designed to contain the DNA sequences defined in vaccinia flanked by restriction sites which could be used to combine the cassettes in any order or combination. Initiator methionines were also designed into each cassette such that inframe fusions could be made at either *EcoRI* or *BamHI* sites. A set of translational stop codons in all three reading frames and an early transcriptional termination signal (9) were also engineered downstream of the inframe fusion site. DNA encoding each cassette was synthesized according to standard techniques and cloned into the appropriate homology vectors (see Figures 3 and 4).

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VACCINATION STUDIES IN SWINE USING RECOMBINANT SWINEPOX VIRUS CONTAINING PSEUDORABIES VIRUS GLYCOPROTEIN GENES:

Young weaned pigs from pseudorabies-free herd are used to test the efficacy of the recombinant swinepox virus containing one or more of the pseudorabies virus glycoprotein genes (SPV/PRV). The piglets are inoculated intramuscularly, intradermally or orally about 10^3 to 10^7 plaque forming units (PFU) of the recombinant SPV/PRV viruses.

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Immunity is determined by measuring PRV serum antibody levels and by challenging the vaccinated pigs with virulent strain of pseudorabies virus. Three to four weeks post-vaccination, both vaccinated and non-vaccinated groups of pigs are challenged with virulent strain of pseudorabies virus (VDL4892). Post challenge, the pigs are observed daily for 14 days for clinical signs of pseudorabies.

20 Serum samples are obtained at the time of vaccination, challenge, and at weekly intervals for two to three weeks post-vaccination and assayed for serum neutralizing antibody.

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CLONING OF BOVINE VIRAL DIARRHEA VIRUS g48 and g53 GENES.

The bovine viral diarrhea g48 and g53 genes were cloned by a PCR CLONING procedure essentially as described by Katz et al. (42) for the HA gene of human influenza. Viral RNA prepared from BVD virus Singer strain grown in Madin-Darby bovine kidney (MDBK) cells was first converted to cDNA utilizing an oligonucleotide primer specific for the target gene. The cDNA was then used as a template for polymerase chain reaction (PCR) cloning (15) of the targeted region. The PCR primers were designed to incorporate restriction sites which permit the cloning of the amplified coding regions into vectors

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containing the appropriate signals for expression in SPV. One pair of oligonucleotides were required for each coding region. The g48 gene coding region from the BVDV Singer strain (49) was cloned using the following primers: 5'-ACGTCGGATCCCTTACCAAACACGTCTTACTCTTGTTTCC-3' for cDNA priming and combined with 5'-ACATAGGATCCCATGGGAGAAAACATAACACAGTGGAACC-3' for PCR. The g53 gene coding region from the BVDV Singer strain (49) was cloned using the following primers: 5'-CGTGGATCCTCAATTACAAGAGGTATCGTCTAC-3' for cDNA priming and combined with 5'-CATAGATCTTGTGGTGCTGTCCGACTTCGCA-3' for PCR. Note that this general strategy is used to clone the coding region of g48 and g53 genes from other strains of BVDV. The DNA fragment for BVDV g 48 was digested with *Bam*HI to yield an 678 bp fragment. The DNA fragment for BVDV g53 was digested with *Bgl*II and *Bam*HI to yield an 1187 bp fragment. The BVDV g48 or g53 DNA fragments were cloned into the *Bam*HI site next to the LP2EP2 promoter of the SPV homology vector to yield homology vectors, 727-78.1 and 738-96, respectively.

CLONING OF BOVINE RESPIRATORY SYNCYTIAL VIRUS FUSION, NUCLEOCAPSID AND GLYCOPROTEIN GENES. The bovine respiratory syncytial virus fusion (F), nucleocapsid (N), and glycoprotein (G) genes were cloned by a PCR CLONING procedure essentially as described by Katz et al. (42) for the HA gene of human influenza. Viral RNA prepared from BRSV virus grown in bovine nasal turbinate (BT) cells was first converted to cDNA utilizing an oligonucleotide primer specific for the target gene. The cDNA was then used as a template for polymerase chain reaction (PCR) cloning (15) of the targeted region. The PCR primers were designed to incorporate restriction sites which permit the cloning of the amplified coding regions into vectors containing the appropriate signals for expression in SPV. One pair of oligonucleotides were required for each coding region. The F gene coding region

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from the BRSV strain 375 (VR-1339) was cloned using the following primers: 5' - TGCAGGATCCTCATTCTAAAGGAAAGATTGTTGAT-3' for cDNA priming and combined with 5' - CTCTGGATCCTACAGCCATGAGGATGATCATCAGC-3' for PCR. The N gene coding region from BRSV strain 375 (VR-1339) was cloned utilizing the following primers: 5' - CGTCGGATCCCTCACAGTTCCACATCATTGTCTTTGGGAT-3' for cDNA priming and combined with 5' - CTTAGGATCCCATGGCTCTTAGCAAGGTCAAATAAATGAC-3' for PCR. The G gene coding region from BRSV strain 375 (VR-1339) was cloned utilizing the following primers: 5' - CGTTGGATCCCTAGATCTGTGTAGTTGATTGATTTGTGTGA-3' for cDNA priming and combined with 5' - CTCTGGATCCTCATAACCATCATCTTAAATTCAAGACATTA-3' for PCR. Note that this general strategy is used to clone the coding region of F, N and G genes from other strains of BRSV. The DNA fragments for BRSV F, N, or G were digested with BamHI to yield 1722 bp, 1173 bp, or 771 bp fragments, respectively. The BRSV F, N, and G DNA fragments were cloned into the BamHI site next to the LP2EP2 promoter of the SPV homology vector to yield homology vectors, 727-20.10, 713-55.37 and 727-20.5, respectively.

25

RNA ISOLATED FROM CONCAVALIN A STIMULATED CHICKEN SPLEEN CELLS. Chicken spleens were dissected from 3 week old SPAFAS hatched chicks, washed, and disrupted through a syringe/needle to release cells. After allowing stroma and debris to settle out, the cells were pelleted and washed twice with PBS. The cell pellet was treated with a hypotonic lysis buffer to lyse red blood cells, and splenocytes were recovered and washed twice with PBS. Splenocytes were resuspended at 5×10^6 cells/ml in RPMI containing 5% FBS and 5 μ g/ml Concanavalin A and incubated at 39° for 48 hours. Total RNA was isolated from the cells using guanidine isothionate lysis reagents

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and protocols from the Promega RNA isolation kit (Promega Corporation, Madison WI). 4 μ g of total RNA was used in each 1st strand reaction containing the appropriate antisense primers and AMV reverse transcriptase (Promega Corporation, Madison WI). cDNA synthesis was performed in the same tube following the reverse transcriptase reaction, using the appropriate sense primers and Vent[®] DNA polymerase (Life Technologies, Inc. Bethesda, MD).

10 **SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES.** When the E.coli β -glucuronidase (uidA) marker gene was incorporated into a recombinant virus the plaques containing recombinants were visualized by a simple assay. The enzymatic substrate was incorporated
15 (300 μ g/ml) into the agarose overlay during the plaque assay. For the uidA marker gene the substrate X-Glucuro Chx (5-bromo-4-chloro-3-indolyl- β -D-glucuronic acid Cyclohexylammonium salt, Biosynth AG) was used. Plaques that expressed active marker enzyme turned blue. The
20 blue plaques were then picked onto fresh ESK-4 cells and purified by further blue plaque isolation. In recombinant virus strategies in which the enzymatic marker gene is removed the assay involves plaque purifying white plaques from a background of parental blue plaques. In both
25 cases viruses were typically purified with three rounds of plaque purification.

HOMOLOGY VECTOR 515-85.1. The plasmid 515-85.1 was constructed for the purpose of inserting foreign DNA into
30 SPV. It contains a unique AccI restriction enzyme site into which foreign DNA may be inserted. When a plasmid, containing a foreign DNA insert at the AccI site, is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing the foreign
35 DNA will result. A restriction map of the DNA insert in homology vector 515-85.1 is given in Figures 3A-3C. It may be constructed utilizing standard recombinant DNA

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techniques (22 and 29), by joining two restriction fragments from the following sources. The first fragment is an approximately 2972 base pair *Hind*III to *Bam*HI restriction fragment of pSP64 (Promega). The second
5 fragment is an approximately 3628 base pair *Hind*III to *Bgl*II restriction sub-fragment of the SPV *Hind*III restriction fragment M (23).

HOMOLOGY VECTOR 520-17.5. The plasmid 520-17.5 was
10 constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* β -galactosidase (*lacZ*) marker gene flanked by SPV DNA. Upstream of the marker gene is an approximately 2149 base pair fragment of SPV DNA. Downstream of the marker gene is an approximately
15 1484 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the marker gene will result. Note that the β -galactosidase (*lacZ*) marker gene is under the control of
20 a synthetic early/late pox promoter. A detailed description of the plasmid is given in Figures 3A-3C. It may be constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the synthetic DNA
25 sequences indicated in Figures 3A-3C. The plasmid vector was derived from an approximately 2972 base pair *Hind*III to *Bam*HI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 2149 base pair *Hind*III to *Acc*I restriction sub-fragment of the SPV *Hind*III
30 restriction fragment M (23). Fragment 2 is an approximately 3006 base pair *Bam*HI to *Pvu*II restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 1484 base pair *Acc*I to *Bgl*II restriction sub-fragment of the SPV *Hind*III fragment M (23).

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HOMOLOGY VECTOR 538-46.16. The plasmid 538-46.16 was constructed for the purpose of inserting foreign DNA into

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SPV. It incorporates an *E. coli* β -galactosidase (*lacZ*) marker gene and the PRV g50 (gD) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter (LP1) and the g50 (gD) gene is under the control of a synthetic early/late pox promoter (EP1LP2). A detailed description of the plasmid is given in 3A-3C. It may be constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in Figures 3A-3C. The plasmid vector was derived from an approximately 2972 base pair *Hind*III to *Bam*HI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 2149 base pair *Hind*III to *Acc*I restriction sub-fragment of the SPV *Hind*III restriction fragment M (23). Fragment 2 is an approximately 3006 base pair *Bam*HI to *Pvu*II restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 1571 base pair *Eco*RI to *Stu*I restriction sub-fragment of the PRV *Bam*HI fragment 7 (21). Note that the *Eco*RI site was introduced in to this fragment by PCR cloning. In this procedure the primers described below were used along with a template consisting of a PRV *Bam*HI #7 fragment subcloned into pSP64. The first primer 87.03 (5' - CGCGAATTCGCTCG CAGCGCTATTGGC-3') sits down on the PRV g50 (gD) sequence (26) at approximately amino acid 3 priming toward the 3' end of the gene. The second primer 87.06 (5' - GTAGGAGTGGCTGCTGAAG-3') sits down on the opposite strand at approximately amino acid 174 priming toward the 5' end of the gene. The PCR product may be digested with *Eco*RI and *Sal*I to produce an approximately

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509 base pair fragment. The approximately 1049 base pair SalI to StuI sub-fragment of PRV BamHI #7 may then be ligated to the approximately 509 base pair EcoRI to SalI fragment to generate the approximately 1558 base pair EcoRI to StuI fragment 3. Fragment 4 is an approximately 1484 base pair AccI to BglII restriction sub-fragment of the SPV HindIII fragment M (23).

10 **HOMOLOGY VECTOR 570-91.41.** The plasmid 570-91.41 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* β -galactosidase (lacZ) marker gene and the PRV gIII (gC) gene flanked by SPV DNA. Upstream of the foreign DNA genes is an
15 approximately 2149 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding
20 for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), and the gIII (gC) gene is under the control of a synthetic early late pox promoter (EP1LP2). A detailed description of the plasmid
25 is given in Figures 5A-5D. It may be constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in 5A-5D. The plasmid vector was derived from an approximately
30 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3002 base pair BamHI to PvuII restriction
35 fragment of plasmid pJF751 (11). Fragment 3 is an approximately 2378 base pair NcoI to NcoI fragment of plasmid 251-41.A, a subfragment of PRV BamHI #2 and #9.

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EcoRI linkers have replaced the *NcoI* and *NcoI* sites at the ends of this fragment. Fragment 4 is an approximately 2149 base pair *AccI* to *HindIII* restriction sub-fragment of the SPV *HindIII* fragment M (23). The *AccI* sites in fragments 1 and 4 have been converted to *PstI* sites using synthetic DNA linkers.

HOMOLOGY VECTOR 570-91.64. The plasmid 570-91.64 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* β -galactosidase (*lacZ*) marker gene and the PRV *gIII* (*gC*) gene flanked by SPV DNA. Upstream of the foreign DNA genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter (LP1), and the *gIII* (*gC*) gene is under the control of a synthetic late early pox promoter (LP2EP2). A detailed description of the plasmid is given in Figures 7A-7D. It may be constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in 5 A-5D. The plasmid vector was derived from an approximately 2972 base pair *HindIII* to *BamHI* restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *BglIII* to *AccI* restriction sub-fragment of the SPV *HindIII* restriction fragment M (23). Fragment 2 is an approximately 3002 base pair *BamHI* to *PvuII* restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 2378 base pair *NcoI* to *NcoI* fragment of plasmid 251-41.A, a subfragment of PRV *BamHI* #2 and #9. *EcoRI* linkers have replaced the *NcoI* and *NcoI* sites at the ends of this fragment.

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Fragment 4 is an approximately 2149 base pair *AccI* to *HindIII* restriction sub-fragment of the SPV *HindIII* fragment M (23). The *AccI* sites in fragments 1 and 4 have been converted to *PstI* sites using synthetic DNA linkers.

HOMOLOGY VECTOR 727-54.60. The plasmid 727-54.60 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* β -galactosidase (*lacZ*) marker gene and the pseudorabies virus (PRV) *gII* (*gB*) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter (LP1), and the PRV *gB* gene is under the control of a synthetic late/early pox promoter (LP2EP2). A detailed description of the plasmid is given in Figures 7A-7D. It may be constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in Figures 7A-7D. The plasmid vector was derived from an approximately 2972 base pair *HindIII* to *BamHI* restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *BglIII* to *AccI* restriction sub-fragment of the SPV *HindIII* restriction fragment M (23). Fragment 2 is an approximately 3500 base pair fragment which contains the coding sequence for the PRV *gB* gene within the *KpnI* C fragment of genomic PRV DNA (21). Fragment 2 contains an approximately 53 base pair synthetic fragment containing the amino terminus of the PRV *gB* gene, an approximately 78 base pair *SmaI* to

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Nhe I fragment from the PRV KpnI C genomic fragment, and an approximately 3370 base pair NheI to EcoRI fragment from the PRV KpnI C genomic fragment (21). Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

10

HOMOLOGY VECTOR 751-07.A1. The plasmid 751-07.A1 was used to insert foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the chicken interferon (cIFN) gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β -galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the cIFN gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1146 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 2 is an approximately 577 base pair EcoRI to BglII fragment coding for the cIFN gene (54) derived by reverse transcription and polymerase chain reaction (PCR) (Sambrook, et al., 1989) of RNA ISOLATED FROM CONCANAVALLIN A STIMULATED CHICKEN SPLEEN CELLS. The antisense primer (6/94.13) used for reverse transcription

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and PCR was 5'-CGACGGATCCGAGGTGCGTTTGGGGCTAAGTGC-3' (SEQ ID NO: 211). The sense primer (6/94.12) used for PCR was 5'-CCACGGATCCAGCACAAACGCGAGTCCCACCATGGCT-3' (SEQ ID NO: 212). The BamHI fragment resulting from reverse transcription and PCR was gel purified and used as a template for a second PCR reaction to introduce a unique EcoRI site at the 5' end and a unique BglII site at the 3' end. The second PCR reaction used primer 6/94.22 (5'-CCACGAATTCGATGGCTGTGCCTGCAAGCCCACAG-3'; SEQ ID NO: 213) at the 5' end and primer 6/94.34 (5'-CGAAGATCTGAGGTGCGTTTGGGGCTAAGTGC-3'; SEQ ID NO: 214) at the 3' end to yield an approximately 577 base pair fragment. The DNA fragment contains the coding sequence from amino acid 1 to amino acid 193 of the chicken interferon protein (54) which includes a 31 amino acid signal sequence at the amino terminus and 162 amino acids of the mature protein encoding chicken interferon. Fragment 3 is an approximately 3002 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2156 base-pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology vector was converted to a unique NotI site.

HOMOLOGY VECTOR 751-56.A1. The plasmid 751-56.A1 was used to insert foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the chicken myelomonocytic growth factor (cMGF) gene flanked by SPV DNA. When this plasmid was used according to the

HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β -galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the cMGF gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining

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restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega).

5 Fragment 1 is an approximately 1146 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 2 is an approximately 640 base pair EcoRI to BamHI fragment coding for the cMGF gene(55) derived by reverse transcription and polymerase chain

10 reaction (PCR) (Sambrook, et al., 1989) of RNA ISOLATED FROM CONCAVALIN A STIMULATED CHICKEN SPLEEN CELLS. The antisense primer (6/94.20) used for reverse transcription and PCR was 5'-CGCAGGATCCGGGGCGTCAGAGGCGGGCGAGGTG-3' (SEQ ID NO: 215). The sense primer (5/94.5) used for PCR

15 was 5'-GAGCGGATCCTGCAGGAGGAGACACAGAGCTG-3' (SEQ ID NO: 216). The BamHI fragment derived from PCR was subcloned into a plasmid and used as a template for a second PCR reaction using primer 6/94.16 (5'-GCGCGAATTCCATGTGCTGCCTCACCCCTGTG-3'; SEQ ID NO: 217) at

20 the 5' end and primer 6/94.20 (5'-CGCAGGATCCGGGGCGTCAGAGGCGGGCGAGGTG-3'; SEQ ID NO: 218) at the 3' end to yield an approximately 640 base pair fragment. The DNA fragment contains the coding sequence from amino acid 1 to amino acid 201 of the cMGF protein

25 (55) which includes a 23 amino acid signal sequence at the amino terminus and 178 amino acids of the mature protein encoding cMGF. Fragment 3 is an approximately 3002 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2156

30 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology vector was converted to a unique NotI site.

35 **HOMOLOGY VECTOR 752-22.1.** The plasmid 752-22.1 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ)

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marker gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a swinepox virus O1L gene promoter. The homology vector also contains the synthetic late/early promoter (LP2EP2) into which a second foreign gene is inserted into a unique BamHI or EcoRI site. A detailed description of the plasmid is given in Figures 10A-10D. It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in Figures 10A-10D. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5'-GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair BamHI to PvuII fragment derived from plasmid pJF751 (49) containing the *E. coli* lacZ gene. Fragment 3 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5'-GCCTGAAGCTTCTAGTACAGTATTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

35 **HOMOLOGY VECTOR 752-29.33.** The plasmid 759.33 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* β -galactosidase (lac Z)

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marker gene and an equine herpesvirus type 1 gB gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a swinepox virus 01L gene promoter and the EHV-1 gB gene is under the control of the late/early promoter (LP2EP2). The LP2EP2 promoter-EHV-1 gB gene cassette was inserted into a NotI site of homology vector 738-94.4. Homology vector 752-29.33 was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5'-GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair BamHI to PvuII fragment derived from plasmid pJF751 (49) containing the *E. coli* lacZ gene. Fragment 3 is the product of a PCR reaction (EcoRI to BamHI) and a restriction fragment (BamHI to PmeI) ligated together to yield an EHV-1 gB gene which is an EcoRI to PmeI fragment approximately 2941 base pairs (979 amino acids) in length. The PCR fragment is an approximately 429 base pair fragment having a synthetic EcoRI site at the 5' end of the gene and a natural BamHI site at the 3' end within the BamHI "a" fragment of EHV-1 genomic DNA. The restriction fragment is an approximately 2512 base pair fragment from BamHI to PmeI

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within the BamHI "I" fragment of EHV-1 genomic DNA. In the procedure to produce the 5' end PCR fragment, the primers described below were used with a template consisting of the EHV-1 BamHI "a" and "i" fragments.

5

The first primer 5/94.3 (5'-CGGAATTCCTCTGGTTCGCCGT-3') sits down on the EHV-1 gB sequence at amino acid number 2 and introduces an EcoRI site at the 5' end of the EHV-1 gB gene and an ATG start codon. The second primer 5/94.4 (5'-GACGGTGGATCCGGTAGGCGGT-3') sits down on the EHV-1 gB sequence at approximately amino acid 144 on the opposite strand to primer 5/94.3 and primes toward the 5' end of the gene. The PCR product was digested with EcoRI and BamHI to yield a fragment 429 base pairs in length corresponding to the 5' end of the EHV-1 gB gene. Fragment 3 consists of the products of the PCR reaction (EcoRI to BamHI) and the restriction fragment (BamHI to PmeI) ligated together to yield an EHV-1 gB gene which is an EcoRI to PmeI fragment approximately 2941 base pairs (979 amino acids) in length. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5'-GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

HOMOLOGY VECTOR 746-94.1. The plasmid 746-94.1 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and an infectious bovine rhinotracheitis virus glycoprotein E (gE) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV,

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a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a swinepox virus O1L gene promoter and the IBRV gE gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. A 1250 base pair EcoRI to BamHI fragment coding for amino acids 1 to 417 of the IBRV gE gene (missing 158 amino acids of the carboxy terminal transmembrane region) was inserted into unique EcoRI and BamHI sites of homology vector 752-22.1 (Figures 10A-10D). The 1250 base pair EcoRI to BamHI fragment was synthesized by polymerase chain reaction (15) using IBRV (Cooper) genomic DNA as a template and primer 10/94.23 (5'-GGGGAATTCAATGCAACCCACCGCGCCGCCCC-3'; SEQ ID NO: 219) at the 5' end of the IBRV gE gene (amino acid 1) and primer 10/94.22 (5'-GGGGGATCCTAGGGCGCGCCCGCCGGCTCGCT-3'; SEQ ID NO: 220) at amino acid 417 of the IBRV gE gene.

HOMOLOGY VECTOR 767-67.3. The plasmid 767-67.3 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and an bovine viral diarrhea virus glycoprotein 53 (BVDV gp53) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a swinepox virus O1L gene promoter and the BVDV gp53 gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30),

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by joining restriction fragments from the following sources with the synthetic DNA sequences. A 1187 base pair BamHI fragment coding for the BVDV gp53 was inserted into the unique BamHI sites of homology vector 752-22.1 (Figures 10A-10D). The 1187 base pair BamHI fragment was synthesized by polymerase chain reaction (15) as described in CLONING OF BOVINE VIRAL DIARRHEA VIRUS gp48 AND gp53 GENES.

10 **HOMOLOGY VECTOR 771-55.11.** The plasmid 771-55.11 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and an bovine viral diarrhea virus glycoprotein 48 (BVDV gp48) gene flanked by SPV DNA.

15 Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV,

20 a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a swinepox virus O1L gene promoter and the BVDV gp48 gene is under the control of the late/early promoter (LP2EP2). It was constructed

25 utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. A 678 base pair BamHI fragment coding for the BVDV gp48 was inserted into the unique BamHI sites of homology vector 752-22.1

30 (Figures 10A-10D). The 678 base pair BamHI fragment was synthesized by polymerase chain reaction (15) as described in CLONING OF BOVINE VIRAL DIARRHEA VIRUS gp48 AND gp53 GENES.

35 **PLASMID 551-47.23.** The plasmid 551-47.23 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates the E. coli β -glucuronidase (β -glu) marker

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gene under the control of a late/early pox promoter (LP2EP2). It is useful to insert the marker gene into sites in the SPV genome to produce a recombinant swinepox virus. It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources. The plasmid vector was derived from an approximately 3005 base pair HindIII restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 1823 base pair EcoRI to SmaI fragment of the plasmid pRAJ260 (Clonetech). Note that the EcoRI and SmaI sites were introduced by PCR cloning. Plasmid 551-47.23 was used to make recombinant swinepox viruses S-SPV-059, S-SPV-060, S-SPV-061, and S-SPV-062.

HOMOLOGY VECTOR 779-94.31. The plasmid 779-94.31 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the pseudorabies virus (PRV) gB (gII) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 538 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1180 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the PRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2). A detailed description of the plasmid is given in Figures 12A-12E. It was constructed utilizing standard recombinant DNA techniques (22, and 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2986 base pair HindIII to PstI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 542 base pair HindIII to BglII restriction sub-fragment of the SPV HindIII restriction

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fragment M (23). Fragment 2 is an approximately 3500 base pair fragment which contains the coding sequence for the PRV gB gene within the KpnI C fragment of genomic PRV DNA (21). Fragment 2 contains an approximately 53 base pair synthetic fragment containing the amino terminus of the PRV gB gene, an approximately 78 base pair SmaI to Nhe I fragment from the PRV KpnI C genomic fragment, and an approximately 3370 base pair NheI to EcoRI fragment from the PRV KpnI C genomic fragment (21). Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 1180 base pair BglII to PstI subfragment of the SPV HindIII fragment M. The BglII sites in fragments 1 and 4 were converted to unique HindIII sites using HindIII linkers.

HOMOLOGY VECTOR 789-41.7. The plasmid 789-41.7 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene, the pseudorabies virus (PRV) gB (gII) gene and the PRV gD (g50) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the PRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2), and the PRV gD gene is under the control of a synthetic early/late pox promoter (EP1LP2). A detailed description of the plasmid is given in Figures 13A-13D. It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid

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vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII
5 restriction fragment M (23). Fragment 2 is an approximately 1552 base pair subfragment of the PRV BamHI #7 fragment which contains the coding sequence of the PRV gD gene from amino acid 3 to amino acid 279. The EcoRI site and the ATG translation start codon are derived from
10 a polymerase chain reaction using a 5' primer with an EcoRI site. The StuI site at the 3' end is normally within the PRV gI gene 3' to the PRV gD gene. The entire open reading frame beginning at the EcoRI site codes for 405 amino acids. Fragment 3 is an approximately 48 base
15 pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 3500 base pair fragment which contains the coding sequence for the PRV gB gene within the KpnI C fragment of genomic PRV DNA(21). Fragment 4 contains an approximately 53 base
20 pair synthetic fragment containing the amino terminus of the PRV gB gene, an approximately 78 base pair SmaI to Nhe I fragment from the PRV KpnI C genomic fragment, and an approximately 3370 base pair NheI to EcoRI fragment from the PRV KpnI C genomic fragment (21). Fragment 5 is
25 an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 6 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites
30 using PstI linkers. The NdeI sites in fragments 3 and 6 were converted to unique HindIII sites using HindIII linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104;) of the SPV HindIII M fragment has been deleted which would span SPV
35 fragments 3 and 6.

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HOMOLOGY VECTOR 789-41.27. The plasmid 789-41.27 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene, the pseudorabies virus (PRV) gB (gII) gene and the PRV gC (gIII) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the PRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2), and the PRV gC gene is under the control of a synthetic early/late pox promoter (EP1LP2). A detailed description of the plasmid is given in Figures 14A-14D. It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1560 base pair HindIII to NdeI subfragment of the SPV HindIII fragment M. Fragment 2 is an approximately 3500 base pair fragment which contains the coding sequence for the PRV gB gene within the KpnI C fragment of genomic PRV DNA(21). Fragment 2 contains an approximately 53 base pair synthetic fragment containing the amino terminus of the PRV gB gene, an approximately 78 base pair SmaI to Nhe I fragment from the PRV KpnI C genomic fragment, and an approximately 3370 base pair NheI to EcoRI fragment from the PRV KpnI C genomic fragment (21). Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 48 base pair AccI to NdeI subfragment of

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the SPV HindIII M fragment. Fragment 5 is an approximately 2378 base pair NcoI to NcoI fragment of plasmid 251-41.A, a subfragment of PRV BamHI #2 and #9. EcoRI linkers have replaced the NcoI sites at the ends of the fragment. Fragment 6 is an approximately 1484 base pair AccI to BglII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The NdeI sites in fragments 1 and 4 were converted to unique HindIII sites using HindIII linkers. The AccI site in fragments 4 and 6 were converted to unique PstI sites using PstI linkers. An approximately 545 base pair NdeI to NdeI (Nucleotides 1560 to 2104;) subfragment of the SPV HindIII M fragment has been deleted which would span SPV fragments 4 and 6.

HOMOLOGY VECTOR 789-41.47. The plasmid 789-41.47 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene, the pseudorabies virus (PRV) gC (gIII) gene and the PRV gD (g50) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the PRV gC gene is under the control of a synthetic early/late pox promoter (EP1LP2), and the PRV gD gene is under the control of a synthetic early/late pox promoter (EP1LP2). A detailed description of the plasmid is given in Figures 15A-15D. It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega).

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Fragment 1 is an approximately 1484 base pair BglII to
AccI restriction sub-fragment of the SPV HindIII
restriction fragment M (23). Fragment 2 is an
approximately 1552 base pair subfragment of the PRV BamHI
5 #7 fragment which contains the coding sequence of the PRV
gD gene from amino acid 3 to amino acid 279. The EcoRI
site and the ATG translation start codon are derived from
a polymerase chain reaction using a 5' primer with an
EcoRI site. The StuI site at the 3' end is normally
10 within the PRV gI gene 3' to the PRV gD gene. The entire
open reading frame beginning at the EcoRI site codes for
405 amino acids. Fragment 3 is an approximately 48 base
pair AccI to NdeI subfragment of the SPV HindIII M
fragment. Fragment 4 is an approximately 3010 base pair
15 BamHI to PvuII restriction fragment of plasmid pJF751
(11). Fragment 5 is an approximately 2378 base pair NcoI
to NcoI fragment of plasmid 251-41.A, a subfragment of
PRV BamHI #2 and #9. EcoRI linkers have replaced the NcoI
sites at the ends of the fragment. Fragment 6 is an
20 approximately 1560 base pair NdeI to HindIII subfragment
of the SPV HindIII fragment M. The AccI sites in
fragments 1 and 3 were converted to unique PstI sites
using PstI linkers. The NdeI sites in fragments 3 and 6
were converted to unique HindIII sites using HindIII
25 linkers. An approximately 545 base pair NdeI to NdeI
subfragment (Nucleotides 1560 to 2104;) of the SPV
HindIII M fragment has been deleted which would span SPV
fragments 3 and 6.

30 **HOMOLOGY VECTOR 789-41.73.** The plasmid 789-41.73 was
constructed for the purpose of inserting foreign DNA into
SPV. It incorporates an E. coli β -galactosidase (lacZ)
marker gene, the pseudorabies virus (PRV) gB (gII) gene,
the PRV gC (gIII) gene and the PRV gD (g50) gene flanked
35 by SPV DNA. Upstream of the foreign genes is an
approximately 1484 base pair fragment of SPV DNA.
Downstream of the foreign genes is an approximately 1560

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base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the PRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2), the PRV gC gene is under the control of a synthetic early/late promoter (EP1LP2), and the PRV gD gene is under the control of a synthetic late/early pox promoter (LP2EP2). A detailed description of the plasmid is given in Figures 16A-16E. It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 1552 base pair subfragment of the PRV BamHI #7 fragment which contains the coding sequence of the PRV gD gene from amino acid 3 to amino acid 279. The EcoRI site and the ATG translation start codon are derived from a polymerase chain reaction using a 5' primer with an EcoRI site. The StuI site at the 3' end is normally within the PRV gI gene 3' to the PRV gD gene. The entire open reading frame beginning at the EcoRI site codes for 405 amino acids. Fragment 3 is an approximately 2378 base pair NcoI to NcoI fragment of plasmid 251-41.A, a subfragment of PRV BamHI #2 and #9. EcoRI linkers have replaced the NcoI sites at the ends of the fragment. Fragment 4 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 5 is an approximately 3500 base pair fragment which contains the coding sequence for the PRV gB gene within the KpnI C fragment of genomic PRV DNA(21). Fragment 5 contains an approximately 53 base pair

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synthetic fragment containing the amino terminus of the PRV gB gene, an approximately 78 base pair SmaI to Nhe I fragment from the PRV KpnI C genomic fragment, and an approximately 3370 base pair NheI to EcoRI fragment from the PRV KpnI C genomic fragment (21). Fragment 6 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 7 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 6 were converted to unique HindIII sites using HindIII linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104;) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 6.

HOMOLOGY VECTOR 791-63.19. The plasmid 791-63.19 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequence. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an

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approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers.

HOMOLOGY VECTOR 791-63.41. The plasmid 791-63.41 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers.

HOMOLOGY VECTOR 796-18.9. The plasmid 796-18.9 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ)

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marker gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic early pox promoter (EP1). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers.

HOMOLOGY VECTOR 783-39.2. The plasmid 783-39.2 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and an bovine viral diarrhea virus glycoprotein 53 (BVDV gp53) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the

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BVDV gp53 gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 1187 base pair BamHI fragment coding for the BVDV gp53. The 1187 base pair BamHI fragment was synthesized by polymerase chain reaction (15) as described in CLONING OF BOVINE VIRAL DIARRHEA VIRUS gp48 AND gp53 GENES. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

HOMOLOGY VECTOR 749-75.78. The plasmid 749-75.78 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the infectious bursal disease virus (IBDV) polymerase gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the IBDV polymerase gene is under the control of a synthetic late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques

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(22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 2700 EcoRI to AscI restriction fragment synthesized by cDNA cloning and polymerase chain reaction (PCR) from an IBDV RNA template. cDNA and PCR primers (5'-CACGAATTCTGACATTTTCAACAGTCCACAGGCGC-3'; 12/93.4) and 5'-GCTGTTGGACATCACGGGCCAGG-3'; 9/93.28) were used to synthesize an approximately 1400 base pair EcoRI to BclI fragment at the 5' end of the IBDV polymerase gene. cDNA and PCR primers (5'-ACCCGGAACATATGGTCAGCTCCAT-3'; 12/93.2) and 5'-GGCGCGCCAGGCGAAGGCCGGGGATACGG-3'; 12/93.3) were used to synthesize an approximately 1800 base pair BclI to AscI fragment at the 3' end of the IBDV polymerase gene. The two fragments were ligated at the BclI site to form the approximately 2700 base pair EcoRI to BclI fragment. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

HOMOLOGY VECTOR 761-75.B18. The plasmid 761-75.B18 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lac Z) marker gene and a feline immunodeficiency virus (FIV) protease (gag) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV,

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a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a swinepox virus 01L gene promoter and the FIV gag gene is under the control of the late/early promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5' GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair BamHI to PvuII fragment derived from plasmid pJF751 (49) containing the E. coli lacZ gene. Fragment 3 is an approximately 1878 base pair EcoRI to BglII restriction fragment synthesized by polymerase chain reaction (PCR) using cDNA from the FIV (PPR strain) (61). The primer (5' GCGTGAATTCCGGGAATGGACAGGGGCGAGAT-3'; 11/94.9) synthesizes from the 5' end of the FIV gag gene, introduces an EcoRI site at the 5' end of the gene and an ATG start codon. The primer (5'-GAGCCAGATCTGCTCTTTTACTTTCCC-3'; 11/94.10) synthesizes from the 3' end of the FIV gag gene. The PCR product was digested with EcoRI and BglII to yield a fragment 1878 base pairs in length corresponding to the FIV gag gene. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5' GCCTGAAGCTTCTAGTACAGTATTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

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HOMOLOGY VECTOR 781-84.C11. The plasmid 781-84.C11 was used to insert foreign DNA into SPV. It incorporates an *E. coli* β -galactosidase (*lacZ*) marker gene and the feline immunodeficiency virus (FIV) envelope (*env*) gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter (LP1) and the FIV *env* gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 3 is an approximately 2564 base pair BamHI to BamHI fragment of the FIV *env* gene (61) synthesized by CLONING WITH THE POLYMERASE CHAIN REACTION. The template for the PCR reaction was FIV strain PPR genomic cDNA (61). The upstream primer 10/93.21 (5'-GCCCGGATCCTATGGCAGAAGGGTTTGCAGC-3';) was synthesized corresponding to the 5' end of the FIV *env* gene starting at nucleotide 6263 of FIV strain PPR genomic cDNA, and the procedure introduced a BamHI site at the 5' end. The BamHI site was destroyed during the cloning of the PCR fragment. The downstream primer 10/93.20 (5'-CCGTGGATCCGGCACTCCATCATTCTCCTC-3';) was synthesized corresponding to the 3' end of the FIV *env* gene starting at nucleotide 8827 of FIV PPR genomic cDNA, and the procedure introduced a BamHI site at the 3' end. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to

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HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology vector was converted to a unique NotI site.

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EXAMPLESExample 1

5 Homology Vector 515-85.1. The homology vector 515-85.1 is a plasmid useful for the insertion of foreign DNA into SPV. Plasmid 515-85.1 contains a unique *AccI* restriction site into which foreign DNA may be cloned. A plasmid containing such a foreign DNA insert may be used
10 according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV to generate a SPV containing the foreign DNA. For this procedure to be successful it is important that the insertion site (*AccI*) be in a region non-essential to the replication of the SPV and
15 that the site be flanked with swinepox virus DNA appropriate for mediating homologous recombination between virus and plasmid DNAs. *AccI* site in homology vector 515-85.1 is used to insert foreign DNA into at least three recombinant SPV (see examples 2-4).

20

In order to define an appropriate insertion site, a library of SPV *HindIII* restriction fragments was generated. Several of these restriction fragments (*HindIII* fragments G, J, and M see Figures 1A-1B) were
25 subjected to restriction mapping analysis. Two restriction sites were identified in each fragment as potential insertion sites. These sites included *HpaI* and *NruI* in fragment G, *BalI* and *XbaI* in fragment J, and *AccI* and *PstI* in fragment M. A β -galactosidase (*lacZ*) marker
30 gene was inserted in each of the potential sites. The resulting plasmids were utilized in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The generation of recombinant virus was determined by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -GALACTOSIDASE
35 ASSAYS. Four of the six sites were found to generate recombinant virus, however the ability of each of these viruses to be purified away from the parental SPV varied

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greatly. In one case virus could not be purified above the level of 1%, in another case virus could not be purified above the level of 50%, and in a third case virus could not be purified above the level of 90%. The inability to purify these viruses indicates instability at the insertion site. This makes the corresponding sites inappropriate for insertion of foreign DNA. However the insertion at one site, the AccI site of Homology vector 515-85.1, resulted in a virus which was easily purified to 100% (see example 2), clearly defining an appropriate site for the insertion of foreign DNA.

The homology vector 515-85.1 was further characterized by DNA sequence analysis. Two regions of the homology vector were sequenced. The first region covers a 599 base pair sequence which flanks the unique AccI site. The second region covers the 899 base pairs upstream of the unique HindIII site. The sequence of the first region codes for an open reading frame (ORF) which shows homology to amino acids 1 to 115 of the vaccinia virus (VV) O1L open reading frame identified by Goebel *et al.*, 1990 (see Figures 2A-2C). The sequence of the second region codes for an open reading frame which shows homology to amino acids 568 to 666 of the same vaccinia virus O1L open reading frame (see Figures 2A-2C). These data suggest that the AccI site interrupts the presumptive VV O1L-like ORF at approximately amino acid 41, suggesting that this ORF codes for a gene non-essential for SPV replication. Goebel *et al.* suggest that the VV O1L ORF contains a leucine zipper motif characteristic of certain eukaryotic transcriptional regulatory proteins, however they indicate that it is not known whether this gene is essential for virus replication.

The DNA sequence located upstream of the VV O1L-like ORF would be expected to contain a swinepox viral promoter.

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This swinepox viral promoter will be useful as the control element of foreign DNA introduced into the swinepox genome.

5 Example 2

S-SPV-003

10 S-SPV-003 is a swinepox virus that expresses a foreign gene. The gene for E.coli β -galactosidase (lacZ gene) was inserted into the SPV 515-85.1 ORF. The foreign gene (lacZ) is under the control of a synthetic early/late promoter (EP1LP2).

15 S-SPV-003 was derived from S-SPV-001 (Kasza strain). This was accomplished utilizing the homology vector 520-17.5 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by
20 the SCREEN FOR RECOMBINANT SPV EXPRESSING β -GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-003. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple
25 passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable and expressing the foreign gene. The assays described here were carried
30 out in VERO cells as well as EMSK cells, indicating that VERO cells would be a suitable substrate for the production of SPV recombinant vaccines. S-SPV-003 has been deposited with the ATCC under Accession No. VR 2335.

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Example 3S-SPV-008

5 S-SPV-008 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ gene) and the gene for pseudorabies virus (PRV) g50 (gD) (26) were inserted into the SPV 515-85.1 ORF. The lacZ gene is under the control of a synthetic late
10 promoter (LP1) and the g50 (gD) gene is under the control of a synthetic early/late promoter (EP1LP2).

S-SPV-008 was derived from S-SPV-001 (Kasza strain). This was accomplished utilizing the homology vector 538-46.16
15 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red
20 plaque purification was the recombinant virus designated S-SPV-008. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds
25 of purification, all plaques observed were blue indicating that the virus was pure, stable and expressing the marker gene.

S-SPV-008 was assayed for expression of PRV specific
30 antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Swine anti-PRV serum was shown to react specifically with S-SPV-008 plaques and not with S-SPV-009 negative control plaques. All S-SPV-008 observed plaques reacted with the swine antiserum
35 indicating that the virus was stably expressing the PRV foreign gene. The black plaque assay was also performed on unfixed monolayers. The SPV plaques on the unfixed

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monolayers also exhibited specific reactivity with swine anti-PRV serum indicating that the PRV antigen is expressed on the infected cell surface.

- 5 To confirm the expression of the PRV g50 (gD) gene product, cells were infected with SPV and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. The
10 swine anti-PRV serum was used to detect expression of PRV specific proteins. The lysate from S-SPV-008 infected cells exhibits a specific band of approximately 48 kd, the reported size of PRV g50 (gD) (35).
- 15 PRV g50 (gD) is the g50 (gD) homologue of HSV-1 (26). Several investigators have shown that VV expressing HSV-1 g50 (gD) will protect mice against challenge with HSV-1 (6 and 34). Therefore the S-SPV-008 should be valuable as a vaccine to protect swine against PRV disease.
- 20 It is anticipated that several other PRV glycoproteins will be useful in the creation of recombinant swinepox vaccines to protect against PRV disease. These PRV glycoproteins include gII (28), gIII (27), and gH (19).
- 25 The PRV gIII coding region has been engineered behind several synthetic pox promoters. The techniques utilized for the creation of S-SPV-008 will be used to create recombinant swinepox viruses expressing all four of these PRV glycoprotein genes. Such recombinant swinepox
30 viruses will be useful as vaccines against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated
35 animals from infected animals. S-SPV-008 has been deposited with the ATCC under Accession No. VR 2339.

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Example 6S-SPV-013

5 S-SPV-013 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for pseudorabies virus gIII (gC) were inserted into the unique *Pst*I restriction site (*Pst*I linkers inserted into a unique *Acc*I site) of the homology
10 vector 570-33.32. The lacZ gene is under the control of the synthetic late promoter (LP1) and the PRV gIII (gC) gene is under the control of the synthetic late early promoter (LP2EP2).

15 S-SPV-013 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 570-91.64 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by
20 the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-013. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple
25 passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

30 S-SPV-013 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal goat anti-PRV gIII (gC) antibody was shown to react specifically with
35 S-SPV-013 plaques and not with S-SPV-001 negative control plaques. All S-SPV-013 observed plaques reacted with the swine anti-PRV serum indicating that the virus was stably

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expressing the PRV foreign gene. The assays described here were carried out in EMSK and VERO cells, indicating that EMSK cells would be a suitable substrate for the production of SPV recombinant vaccines.

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To confirm the expression of the PRV gIII (gC) gene product, cells were infected with SPV and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Polyclonal goat anti-PRV gIII (gC) antibody was used to detect expression of PRV specific proteins. The lysate from S-SPV-013 infected cells exhibits two specific bands which are the reported size of PRV gIII (gC) (37)—a 92 kd mature form and a 74 kd pre-Golgi form.

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15

Recombinant-expressed PRV gIII (gC) has been shown to elicit a significant immune response in mice and swine (37, 38). Furthermore, when gIII (gC) is coexpressed with gII (gB) or g50 (gD), significant protection from challenge with virulent PRV is obtained. (39) Therefore S-SPV-013 is valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals. S-SPV-013 has been deposited with the ATCC under Accession No. 2418.

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Protection against Aujeszky's disease using recombinant swinepox virus vaccines S-SPV-008 and S-SPV-013.

A vaccine containing S-SPV-008 and S-SPV-013 (1 x 10⁶PFU/ml) (2ml of a 1:1 mixture of the two viruses) was given to two groups of pigs (5 pigs per group) by intradermal inoculation or by oral/pharyngeal spray. A

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control group of 5 pigs received S-SPV-001 by both intradermal and oral/pharyngeal inoculation. Pigs were challenged three weeks post-vaccination with virulent PRV, strain 4892, by intranasal inoculation. The table presents a summary of clinical responses. The data support an increase in protection against Aujeszky's disease in the S-SPV-008/S-SPV-013 vaccinates compared to the S-SPV-001 vaccinate controls.

10	Vaccine	Route of inoculation	Post-challenge	Post-challenge	Post-challenge Group average: (Days of clinical signs)
			Respiratory Signs: (# with signs/total number)	CNS signs: (# with signs/total number)	
	S-SPV-008 + S-SPV-013	Intradermal	3/5	0/5	2.6
	S-SPV-008 + S-SPV-013	Oral/pharyngeal	3/5	0/5	2.2
15	S-SPV-001 (Control)	Intradermal + Oral/Pharyngeal	5/5	2/5	7.8

Example 7

20 S-SPV-015

S-SPV-015 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for pseudorabies virus (PRV) gII (gB) were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the PRV gB gene is under the control of the synthetic late/early promoter (LP2EP2).

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S-SPV-015 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 727-54.60 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-015. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-015 was assayed for expression of PRV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-PRV serum was shown to react specifically with S-SPV-015 plaques and not with S-SPV-001 negative control plaques. All S-SPV-015 observed plaques reacted with the antiserum indicating that the virus was stably expressing the PRV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the PRV gII gene product, cells were infected with SPV-015 and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Polyclonal swine anti-PRV serum was used to detect expression of PRV specific proteins. The lysate from S-SPV-015 infected cells exhibited bands corresponding to 120 kd, 67 kd and 58 kd, which are the expected size of the PRV gII glycoprotein.

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S-SPV-015 is useful as a vaccine in swine against pseudorabies virus. A superior vaccine is formulated by combining S-SPV-008 (PRV g50), S-SPV-013 (PRV gIII), and S-SPV-015 for protection against pseudorabies in swine.

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Therefore S-SPV-015 should be valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals. S-SPV-015 has been deposited with the ATCC under Accession No. 2466.

15 Example 8

Recombinant swinepox virus expressing more than one pseudorabies virus (PRV) glycoproteins, which can elicit production of neutralizing antibodies against pseudorabies virus, is constructed in order to obtain a recombinant swinepox virus with enhanced ability to protect against PRV infection than that which can be obtained by using a recombinant swinepox virus expressing only one of those PRV glycoproteins.

25

There are several examples of such recombinant swinepox virus expressing more than one PRV glycoproteins: a recombinant swinepox virus expressing PRV g50 (gD) and gIII (gC), a recombinant swinepox virus expressing PRV g50 (gD) and gII (gB); a recombinant swinepox virus expressing PRV gII (gB) and gIII (gC); and a recombinant swinepox virus expressing PRV g50 (gD), gIII (gC) and gII (gB). Each of the viruses cited above is also engineered to contain and express *E. coli* β -galactosidase (lac Z) gene, which will facilitate the cloning of the recombinant swinepox virus.

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Listed below are three examples of a recombinant swinepox virus expressing PRV g50 (gD), PRV gIII (gC), PRV gII (gB) and *E. coli* β -galactosidase (lacZ):

- 5 a) Recombinant swinepox virus containing and
 expressing PRV g50 (gD) gene, PRV gIII (gC) gene,
 PRV gII (gB) gene and lacZ gene. All four genes are
 inserted into the unique *AccI* restriction
10 endonuclease site within the *HindIII* M fragment of
 the swinepox virus genome. PRV g50 (gD) gene is
 under the control of a synthetic early/late promoter
 (EP1LP2), PRV gIII (gC) gene is under the control of
 a synthetic early promoter (EP2), PRV gII (gB) gene
 is under the control of a synthetic late/early
15 promoter (LP2EP2) and lacZ gene is under the control
 of a synthetic late promoter (LP1).
- b) Recombinant swinepox virus containing and
 expressing PRV g50 (gD) gene, PRV gIII (gC) gene,
20 PRV gII (gB) gene and lacZ gene. All four genes are
 inserted into the unique *AccI* restriction
 endonuclease site within the *HindIII* M fragment of
 the swinepox virus genome. PRV g50 (gD) gene is
 under the control of a synthetic early/late promoter
25 (EP1LP2), PRV gIII (gC) gene is under the control of
 a synthetic early/late promoter (EP1LP2), PRV gII
 (gB) gene is under the control of a synthetic
 late/early promoter (LP2EP2) and lacZ gene is under
 the control of a synthetic late promoter (LP1).
- 30 c) Recombinant swinepox virus containing and
 expressing PRV g50 (gD) gene, PRV gIII (gC) gene,
 PRV gII (gB) gene and lacZ gene. All four genes are
 inserted into the unique *AccI* restriction
35 endonuclease site within the *HindIII* M fragment of
 the swinepox virus genome. PRV g50 (gD) gene is
 under the control of a synthetic early/late promoter

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(EP1LP2), PRV gIII (gC) gene is under the control of a synthetic late/early promoter (LP2EP2), PRV gII (gB) gene is under the control of a synthetic late/early promoter (LP2EP2) and lacZ gene is under the control of a synthetic late promoter (LP1).

Protection against Aujeszky's disease using recombinant swinepox virus vaccines S-SPV-008, S-SPV-013 and S-SPV-015.

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A vaccine containing S-SPV-008, S-SPV-013, or S-SPV-015 (2 ml of 1×10^7 PFU/ml of the virus) or a mixture of S-SPV-008, S-SPV-013, and S-SPV-015 (2ml of a 1:1:1 mixture of the three viruses; 1×10^7 PFU/ml) was given to four groups of pigs (5 pigs per group) by intramuscular inoculation. A control group of 5 pigs received S-SPV-001 by intramuscular inoculation. Pigs were challenged four weeks post-vaccination with virulent PRV, strain 4892, by intranasal inoculation. The pigs were observed daily for 14 days for clinical signs of pseudorabies, and the table presents a summary of clinical responses. The data show that pigs vaccinated with S-SPV-008, S-SPV-013, or S-SPV-015 had partial protection and pigs vaccinated with the combination vaccine S-SPV-008/S-SPV-013/S-SPV-015 had complete protection against Aujeszky's disease caused by pseudorabies virus compared to the S-SPV-001 vaccinate controls.

	Vaccine	Route of inoculation	Post-challenge	Post-challenge	Post-challenge
			Respiratory Signs: (# with signs/total number)	CNS signs: (# with signs/total number)	Group average: (Days of clinical signs)
30	S-SPV-008	Intramuscular	2/5	2/5	2.0
	S-SPV-013	Intramuscular	1/5	0/5	0.4

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	S-SPV-015	Intramuscular	3/5	0/5	1.0
	S-SPV-008 +	Intramuscular	0/5	0/5	0.0
	S-SPV-013 +				
	S-SPV-015				
5	S-SPV-001	Intramuscular	5/5	2/5	3.6
	(Control)				

Example 17

10

The development of vaccines utilizing the swinepox virus to express antigens from various disease causing microorganisms can be engineered.

15 **TRANSMISSIBLE GASTROENTERITIS VIRUS**

The major neutralizing antigen of the transmissible gastroenteritis virus (TGE), glycoprotein 195, for use in the swinepox virus vector has been cloned. The clone of the neutralizing antigen is disclosed in U.S. Serial No. 078,519, filed July 27, 1987. It is contemplated that the procedures that have been used to express PRV g50 (gD) in SPV and are disclosed herein are applicable to TGE.

25 **PORCINE PARVOVIRUS**

The major capsid protein of the porcine (swine) parvovirus (PPV) was cloned for use in the swinepox virus vector. The clone of the capsid protein is disclosed in U.S. Patent No. 5,068,192 issued November 26, 1991. It is contemplated that the procedures that have been used to express PRV g50 (gD) in SPV and are disclosed herein are applicable to PPV.

SWINE ROTAVIRUS

35 The major neutralizing antigen of the swine rotavirus, glycoprotein 38, was cloned for use in the swinepox virus vector. The clone of glycoprotein 38 is disclosed

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in U.S. Patent No. 5,068,192 issued November 26, 1991. It is contemplated that the procedures that have been used to express PRV g50 (gD) in SPV and are disclosed herein are applicable to SRV.

5

HOG CHOLERA VIRUS

The major neutralizing antigen of the bovine viral diarrhea (BVD) virus was cloned as disclosed in U.S. Serial No. 225,032, filed July 27, 1988. Since the BVD and hog cholera viruses are cross protective (31), the BVD virus antigen has been targeted for use in the swinepox virus vector. It is contemplated that the procedures that have been used to express PRV g50 (gD) in SPV and are disclosed herein are applicable to BVD virus.

15

SERPULINA HYODYSENTERIAE

A protective antigen of *Serpulina hyodysenteriae* (3), for use in the swinepox virus vector has been cloned. It is contemplated that the procedures that have been used to express PRV g50 in SPV and are disclosed herein are also applicable to *Serpulina hyodysenteriae*.

20

Antigens from the following microorganisms may also be utilized to develop animal vaccines: swine influenza virus, foot and mouth disease virus, African swine fever virus, hog cholera virus, *Mycoplasma hyopneumoniae*, porcine reproductive and respiratory syndrome/swine infertility and respiratory syndrome (PRRS/SIRS).

25

30

Antigens from the following microorganisms may also be utilized for animal vaccines: 1) canine - herpesvirus, canine distemper, canine adenovirus type 1 (hepatitis), adenovirus type 2 (respiratory disease), parainfluenza, *Leptospira canicola*, icterohemorrhagia, parvovirus, coronavirus, *Borrelia burgdorferi*, canine herpesvirus,

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Bordetella bronchiseptica, *Dirofilaria immitis* (heartworm) and rabies virus. 2) Feline - Fiv gag and env, feline leukemia virus, feline immunodeficiency virus, feline herpesvirus, feline infectious peritonitis virus, canine herpesvirus, canine coronavirus, canine parvovirus, parasitic diseases in animals (including *Dirofilaria immitis* in dogs and cats), equine infectious anemia, *Streptococcus equi*, coccidia, emeria, chicken anemia virus, *Borrelia bergdorferi*, bovine coronavirus, *Pasteurella haemolytica*.

Example 24

15

Homology Vector 738-94.4

Homology Vector 738-94.4 is a swinepox virus vector that expresses one foreign gene. The gene for *E. coli* β -galactosidase (lacZ) was inserted into the the O1L open reading frame. The lacZ gene is under the control of the O1L promoter. The homology vector 738-94.4 contains a deletion of SPV DNA from nucleotides 1679 to 2452 (SEQ ID NO: 189) which deletes part of the O1L ORF.

The upstream SPV sequences were synthesized by polymerase chain reaction using DNA primers 5'-GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with BglII and SphI ends. The O1L promoter is present on this fragment. The downstream SPV sequences were synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5'-GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

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A recombinant swinepox virus was derived utilizing homology vector 738-94.4 and S-SPV-001 (Kasza strain) in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was
5 screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification is the recombinant virus. This virus is assayed for β -galactosidase expression, purity, and insert stability by multiple
10 passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign gene. Recombinant swinepox
15 viruses derived from homology vector 738-94.4 are utilized as an expression vector to express foreign antigens and as a vaccine to raise a protective immune response in animals to foreign genes expressed by the recombinant swinepox virus. Other promoters in addition
20 to the O1L promoter are inserted into the deleted region including LP1, EP1LP2, LP2EP2, HCMV immediate early, and one or more foreign genes are expressed from these promoters.

25 Example 24B

Homology Vector 752-22.1 is a swinepox virus vector that is utilized to express two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) was inserted into
30 the the O1L open reading frame. The lacZ gene is under the control of the O1L promoter. A second foreign gene is expressed from the LP2EP2 promoter inserted into an EcoRI or BamHI site following the LP2EP2 promoter sequence. The homology vector 752-22.1 contains a
35 deletion of SPV DNA from nucleotides 1679 to 2452 (SEQ ID NO: 189) which deletes part of the O1L ORF. The homology vector 752-22.1 was derived from homology

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vector 738-94.4 by insertion of the LP2EP2 promoter fragment (see Materials and Methods). The homology vector 752-22.1 is further improved by placing the lacZ gene under the control of the synthetic LP1 promoter.

5 The LP1 promoter results in higher levels of lacZ expression compared to the SPV O1L promoter

Example 25

10 S-SPV-041:

S-SPV-041 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for equine herpesvirus type 1 glycoprotein B (gB) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1679 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox O1L promoter, and the EHV-1 gB gene is under the control of the

15

20 synthetic late/early promoter (LP2EP2).

S-SPV-041 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 752-29.33 (see Materials and Methods) and virus S-SPV-

25 001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant

30 virus designated S-SPV-041. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all

35 plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

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S-SPV-041 is useful as a vaccine in horses against EHV-1 infection and is useful for expression of EHV-1 glycoprotein B.

5 S-SPV-045:

S-SPV-045 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for infectious bovine
10 rhinotracheitis virus glycoprotein E (gE) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1679 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox O1L promoter, and the IBRV gE gene is under
15 the control of the synthetic late/early promoter (LP2EP2).

S-SPV-045 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector
20 746-94.1 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUGAL AND CPRG ASSAYS). The final
25 result of red plaque purification was the recombinant virus designated S-SPV-045. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods.
30 After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-045 is useful for expression of IBRV glycoprotein
35 E.

S-SPV-049:

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S-SPV-049 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for bovine viral diarrhea virus glycoprotein 48 (gp48) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1679 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox O1L promoter, and the BVDV gp48 gene is under the control of the synthetic late/early promoter (LP2EP2).

10

S-SPV-049 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 771-55.11 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-049. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

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S-SPV-049 is useful as a vaccine in cattle against BVDV infection and is useful for expression of BVDV glycoprotein 48.

S-SPV-050:

S-SPV-050 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for the bovine viral diarrhea virus glycoprotein 53 (gp53) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF;

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Deletion of nucleotides 1679 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox O1L promoter, and the IBRV gE gene is under the control of the synthetic late/early promoter (LP2EP2).

5

S-SPV-050 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 767-67.3 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
10 GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-050. This virus was assayed for
15 β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus
20 was pure, stable, and expressing the foreign gene.

S-SPV-050 is useful as a vaccine in cattle against BVDV infection and is useful for expression of BVDV glycoprotein 53.

25

Example 26

Recombinant swinepox virus, S-SPV-042 or S-SPV-043, expressing chicken interferon (cIFN) or chicken
30 myelomonocytic growth factor (cMGF), respectively, are useful to enhance the immune response when added to vaccines against diseases of poultry. Chicken myelomonocytic growth factor (cMGF) is homologous to mammalian interleukin-6 protein, and chicken interferon
35 (cIFN) is homologous to mammalian interferon. When used in combination with vaccines against specific avian diseases, S-SPV-042 and S-SPV-043 provide enhanced

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mucosal, humoral, or cell mediated immunity against avian disease-causing viruses including, but not limited to, Marek's disease virus, Newcastle disease virus, infectious laryngotracheitis virus, infectious
5 bronchitis virus, infectious bursal disease virus.

Example 26A

S-SPV-042:

10

S-SPV-042 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for chicken interferon (cIFN) were inserted into the SPV 617-48.1 ORF (a unique NotI
15 restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the cIFN gene is under the control of the synthetic late/early promoter (LP2EP2).

20

S-SPV-042 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 751-07.A1 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
25 GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-042. This virus was assayed for
30 β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus
35 was pure, stable, and expressing the foreign gene.

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S-SPV-042 has interferon activity in cell culture. Addition of S-SPV-042 conditioned media to chicken embryo fibroblast (CEF) cell culture inhibits infection of the CEF cells by vesicular stomatitis virus or by herpesvirus of turkeys. S-SPV-042 is useful to enhance the immune response when added to vaccines against diseases of poultry.

Example 26B

10

S-SPV-043:

S-SPV-043 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for chicken myelomonocytic growth factor (cMGF) were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the cMGF gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-043 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 751-56.A1 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-043. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

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S-SPV-043 is useful to enhance the immune response when added to vaccines against diseases of poultry.

Example 27

5

Insertion into a non-essential site in the 2.0 kb HindIII to BglII region of the swinepox virus HindIII M fragment.

- 10 A 2.0 kb HindIII to BglII region of the swinepox virus HindIII M fragment is useful for the insertion of foreign DNA into SPV. The foreign DNA is inserted into a unique BglII restriction site in the region Nucleotide 540 of SEQ ID NOs: 195). A plasmid
15 containing a foreign DNA insert is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV to generate an SPV containing the foreign DNA. For this procedure to be successful, it is important that the insertion site be in a region non-
20 essential to the replication of the SPV and that the site be flanked with swinepox virus DNA appropriate for mediating homologous recombination between virus and plasmid DNAs. The unique BglII restriction site in the 2.0 kb HindIII to BglII region of the swinepox virus
25 HindIII M fragment is located within the coding region of the SPV I4L open reading frame. The I4L ORF has sequence similarity to the vaccinia virus and smallpox virus ribonucleotide reductase (large subunit) gene (56-58). The ribonucleotide reductase (large subunit)
30 gene is non-essential for DNA replication of vaccinia virus and is an appropriate insertion site in swinepox virus.

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Example 28S-SPV-047

5 S-SPV-047 is a swinepox virus that expresses two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for pseudorabies virus gB (gII) were inserted into a unique HindIII site (HindIII linker inserted into the BglII restriction endonuclease
10 site within the 2.0 kb BglII to HindIII subfragment of the HindIII M fragment.) The BglII insertion site is within the SPV I4L open reading frame which has significant homology to the vaccinia virus ribonucleoside-diphosphate reductase gene. The lacZ
15 gene is under the control of the synthetic late promoter (LP1), and the PRV gB (gII) gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-047 was derived from S-SPV-001 (Kasza Strain).
20 This was accomplished utilizing the homology vector 779-94.31 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING
25 β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-047. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue
30 plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

35 S-SPV-047 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-

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PRV serum was shown to react specifically with S-SPV-047 plaques and not with S-SPV-001 negative control plaques. All S-SPV-047 observed plaques reacted with the swine anti-PRV serum indicating that the virus was stably expressing the PRV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

10

To confirm the expression of the PRV gB gene product, cells were infected with S-SPV-047 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRV serum was used to detect expression of PRV specific proteins. The cell lysate and supernatants from S-SPV-047 infected cells exhibited bands corresponding to 120 kD, 67 kD and 58 kD, which are the expected size of the PRV glycoprotein B.

SPV recombinant-expressed PRV gB has been shown to elicit a significant immune response in swine (37, 38; See example 8). Furthermore, PRV gB is expressed in recombinant SPV, significant protection from challenge with virulent PRV is obtained. (See Examples 6 and 8) Therefore S-SPV-047 is valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals.

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S-SPV-052

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S-SPV-052 is a swinepox virus that expresses three foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for pseudorabies virus gB (gII) were inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104;) of the SPV HindIII M fragment has been deleted). The gene for PRV gD (g50) was inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The lacZ gene is under the control of the synthetic late promoter (LP1), the PRV gB (gII) gene is under the control of the synthetic late/early promoter (LP2EP2), and the PRV gD (g50) gene is under the control of the synthetic early/late promoter (EP1LP2).

S-SPV-052 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 789-41.7 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 052. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-052 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-PRV serum was shown to react specifically with S-SPV-

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S-SPV-053 is a swinepox virus that expresses three foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for pseudorabies virus gB (gII) were inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104;) of the SPV HindIII M fragment has been deleted). The gene for PRV gC (gIII) was inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The lacZ gene is under the control of the synthetic late promoter (LP1), the PRV gB (gII) gene is under the control of the synthetic late/early promoter (LP2EP2), and the PRV gC (gIII) gene is under the control of the synthetic early/late promoter (EP1LP2).

S-SPV-053 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 789-41.27 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 053. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-053 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-PRV serum was shown to react specifically with S-SPV-

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- 053 plaques and not with S-SPV-001 negative control plaques. All S-SPV-053 observed plaques reacted with the swine anti-PRV serum indicating that the virus was stably expressing the PRV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.
- 10 To confirm the expression of the PRV gB and gC gene products, cells were infected with S-SPV-053 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRV serum was used to detect expression of PRV specific proteins. The cell lysate and supernatants from S-SPV-053 infected cells exhibited bands corresponding to 120 kD, 67 kD and 58 kD, which are the expected size of the PRV glycoprotein B; and a 92 kD which is the expected size of the PRV glycoprotein C.
- 25 SPV recombinant-expressed PRV gB and gC has been shown to elicit a significant immune response in swine (37, 38; See example 8). Furthermore, PRV gB and gC are expressed in recombinant SPV, significant protection from challenge with virulent PRV is obtained. (See Examples 6 and 8) Therefore S-SPV-053 is valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals.

S-SPV-054

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S-SPV-054 is a swinepox virus that expresses three foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for pseudorabies virus gC (gIII) were inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104;) of the SPV HindIII M fragment has been deleted). The gene for PRV gD (g50) was inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The lacZ gene is under the control of the synthetic late promoter (LP1), the PRV gC (gIII) gene is under the control of the synthetic early/late promoter (EP1LP2), and the PRV gD (g50) gene is under the control of the synthetic early/late promoter (EP1LP2).

S-SPV-054 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 789-41.47 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 054. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-054 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-

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PRV serum was shown to react specifically with S-SPV-054 plaques and not with S-SPV-001 negative control plaques. All S-SPV-054 observed plaques reacted with the swine anti-PRV serum indicating that the virus was stably expressing the PRV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the PRV gC and gD gene products, cells were infected with S-SPV-054 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRV serum was used to detect expression of PRV specific proteins. The cell lysate and supernatants from S-SPV-054 infected cells exhibited a band corresponding to 92 kD which is the expected size of the PRV glycoprotein C and a 48 kD which is the expected size of the PRV glycoprotein D.

SPV recombinant-expressed PRV gC and gD has been shown to elicit a significant immune response in swine (37, 38; See example 8). Furthermore, PRV gC and gD are expressed in recombinant SPV, significant protection from challenge with virulent PRV is obtained. (See Examples 6 and 8) Therefore S-SPV-054 is valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals.

S-SPV-055

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S-SPV-055 is a swinepox virus that expresses four foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for pseudorabies virus gB (gII) were inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104;) of the SPV HindIII M fragment has been deleted). The gene for PRV gD (g50) and PRV gC (gIII) were inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The lacZ gene is under the control of the synthetic late promoter (LP1), the PRV gB (gII) gene is under the control of the synthetic late/early promoter (LP2EP2), the PRV gD (g50) gene is under the control of the synthetic late/early promoter (LP2EP2), and the PRV gC (gIII) gene is under the control of the synthetic early/late promoter (EP1LP2).

S-SPV-055 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 789-41.73 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 055. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-055 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE

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EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-PRV serum was shown to react specifically with S-SPV-055 plaques and not with S-SPV-001 negative control plaques. All S-SPV-055 observed plaques reacted with the swine anti-PRV serum indicating that the virus was stably expressing the PRV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the PRV gB, gC and gD gene products, cells were infected with S-SPV-055 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRV serum was used to detect expression of PRV specific proteins. The cell lysate and supernatants from S-SPV-055 infected cells exhibited a bands corresponding to 120 kD, 67 kD, and 58 kD which is the expected size of the PRV glycoprotein B; a 92 kD which is the expected size of the PRV glycoprotein C; and a 48 kD which is the expected size of the PRV glycoprotein D

SPV recombinant-expressed PRV gB, gC and gD has been shown to elicit a significant immune response in swine (37, 38; See example 8). Furthermore, PRV gB, gC and gD are expressed in recombinant SPV, significant protection from challenge with virulent PRV is obtained. (See Examples 6 and 8) Therefore S-SPV-055 is valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gX or gI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI

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HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals.

Example 29

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S-SPV-059

S-SPV-059 is a swinepox virus that expresses one foreign gene. The gene for E. coli B-glucuronidase (uidA) was inserted into the unique EcoRI restriction site in the SPV B18R open reading frame within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic late/early promoter (LP2EP2). Partial sequence of the SPV 3.2 kb region of the SPV 6.5 kb HindIII K fragment indicates three potential open reading frames. The SPV B18R ORF shows sequences homology to the vaccinia virus B18R gene, 77.2K protein from rabbit fibroma virus, vaccinia virus C19L/B25R ORF and an ankyrin repeat region from a human brain variant. The B18R gene codes for a soluble interferon receptor with high affinity and broad specificity. The SPV B4R open reading frame shows sequence homology to the T5 protein of rabbit fibroma virus.

25 S-SPV-059 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 796-50.31 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. Homology vector 796-50.31 was generated by insertion of a blunt ended NotI fragment containing the LP2EP2 promoter uidA cassette from plasmid 551-47.23 (see Materials and Methods) into a unique EcoRI site (blunt ended) in the SPV 6.5 kb HindIII K fragment, (Figure 29B). The transfection stock was screened by the SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES. The final result of blue plaque purification was the recombinant virus designated S-SPV-059. This virus

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was assayed for B-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-059 has been purified and expresses the foreign gene, E. coli uidA, indicating that the EcoRI site within the 6.5 kb HindIII K fragment is a stable insertion site for foreign genes. Recombinant swinepox virus utilizing this insertion site is useful for expression of foreign antigen genes, as a vaccine against disease or as an expression vector to raise antibodies to the expressed foreign gene.

S-SPV-060

S-SPV-060 is a swinepox virus that expresses one foreign gene. The gene for E. coli B-glucuronidase (uidA) was inserted into the unique EcoRV restriction site within the SPV HindIII N genomic fragment. The uidA gene is under the control of the synthetic late/early promoter (LP2EP2). Partial sequence of the SPV 3.2 kb HindIII N fragment (SEQ ID NO.) indicates two potential open reading frames. The SPV I7L ORF shows sequences homology to protein I7 of vaccinia virus. The SPV I4L open reading frame shows sequence homology to the ribonucleoside diphosphate reductase gene of vaccinia virus. Two potential open reading frames I5L and I6L, between I4L and I7L ORF are of unknown function.

S-SPV-060 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 796-71.31 and virus S-SPV-001 in the HOMOLOGOUS

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RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. Homology vector 796-71.31 was generated by insertion of a blunt ended NotI fragment containing the LP2EP2 promoter uidA cassette from plasmid 551-47.23 (see Materials and Methods) into a unique EcoRV site in the SPV 3.2 kb HindIII N fragment (Figure 11A). The transfection stock was screened by the SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES. The final result of blue plaque purification is the recombinant virus designated S-SPV-060. This virus is assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-SPV-060 is purified and expresses the foreign gene, *E. coli uidA*, indicating that the EcoRI site within the 3.2 kb HindIII N fragment is a stable insertion site for foreign genes. Recombinant swinepox virus utilizing this insertion site is useful for expression of foreign antigen genes, as a vaccine against disease or as an expression vector to raise antibodies to the expressed foreign gene.

S-SPV-061

S-SPV-061 is a swinepox virus that expresses one foreign gene. The gene for *E. coli* β -glucuronidase (*uidA*) was inserted into the unique SnaBI restriction site within the SPV HindIII N genomic fragment. The *uidA* gene is under the control of the synthetic late/early promoter (LP2EP2). Partial sequence of the SPV 3.2 kb HindIII N fragment indicates two potential open reading frames. The SPV I7L ORF shows sequence

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homology to protein 17 of vaccinia virus. The SPV I4L open reading frame shows sequence homology to the ribonucleoside diphosphate reductase gene of vaccinia virus. Two potential open reading frames I5L and I6L, between I4L ORF and I7L ORF are of unknown function.

S-SPV-061 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 796-71.41 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. Homology vector 796-71.41 was generated by insertion of a blunt ended NotI fragment containing the LP2EP2 promoter uidA cassette from plasmid 551-47.23 (see Materials and Methods) into a unique SnaBI site in the SPV 3.2 kb HindIII N fragment. The transfection stock was screened by the SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES. The final result of blue plaque purification is the recombinant virus designated S-SPV-061. This virus is assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-SPV-061 is purified and expresses the foreign gene, E. coli uidA, indicating that the SnaBI site within the 3.2 kb HindIII N fragment is a stable insertion site for foreign genes. Recombinant swinepox virus utilizing this insertion site is useful for expression of foreign antigen genes, as a vaccine against disease or as an expression vector to raise antibodies to the expressed foreign gene.

S-SPV-062

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S-SPV-062 is a swinepox virus that expresses one foreign gene. The gene for *E. coli* β -glucuronidase (*uidA*) was inserted into the unique *Bgl*III restriction site within the SPV *Hind*III N genomic fragment (Figure 11A). The *uidA* gene is under the control of the synthetic late/early promoter (LP2EP2). Partial sequence of the SPV 3.2 kb *Hind*III N fragment indicates two potential open reading frames. The SPV I7L ORF shows sequence homology to protein 17 of vaccinia virus. The SPV I4L open reading frame shows sequence homology to the ribonucleoside diphosphate reductase gene of vaccinia virus. Two potential open reading frames I5L and I6L, between I4L ORF and I7L ORF are of unknown function.

S-SPV-062 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 796-71.51 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. Homology vector 796-71.51 was generated by insertion of a blunt ended *Not*I fragment containing the LP2EP2 promoter *uidA* cassette from plasmid 551-47.23 (see Materials and Methods) into a unique *Bgl*III site in the SPV 3.2 kb *Hind*III N fragment. The transfection stock was screened by the SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES. The final result of blue plaque purification is the recombinant virus designated S-SPV-062. This virus is assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-SPV-062 is purified and expresses the foreign gene, *E. coli uidA*, indicating that the *Bgl*III site within the

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3.2 kb HindIII N fragment is a stable insertion site for foreign genes. Recombinant swinepox virus utilizing this insertion site is useful for expression of foreign antigen genes, as a vaccine against disease or as an expression vector to raise antibodies to the expressed foreign gene.

Example 30:

10 Recombinant swinepox virus expressing E coli β -galactosidase (lacZ) under the control of a synthetic early or synthetic late pox promoter.

15 Three recombinant swinepox viruses, S-SPV-056, S-SPV-057, and S-SPV-058 expressing E coli β -galactosidase (lacZ) under the control of a synthetic pox promoter, LP1, LP2, and EP1, respectively, have been constructed.

20 S-SPV-056 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 791-63.19 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). S-SPV-057 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 791-63.41 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). S-SPV-058 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 796-18.9 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by

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the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification were the recombinant viruses designated S-SPV-056, S-SPV-057 and S-SPV-058.

5 The viruses were assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were

10 blue indicating that the virus was pure, stable, and expressing the foreign gene.

Recombinant swinepox virus expresses a foreign gene such as E. coli β -galactosidase in a human cell line

15 but does not replicate in the human cell line. To optimize expression of the foreign gene, S-SPV-056, S-SPV-057 and S-SPV-058 are used to compare optimal expression levels of E. coli β -galactosidase under the control of early or late synthetic pox viral promoters.

20 The human cell lines in which expression of recombinant swinepox virus has been detected include, but are not limited to 143B (osteosarcoma), A431 (epidermoid carcinoma), A549 (lung carcinoma), Capan-1 (liver carcinoma), CF500 (foreskin fibroblasts), Chang Liver

25 (liver), Detroit (down's foreskin fibroblasts), HEL-199 (embryonic lung), HeLa (cervical carcinoma), HEp-2 (epidermal larynx carcinoma), HISM (intestinal smooth muscle), HNK (neonatal kidney), MRC-5 (embryonic lung), NCI-H292 (pulmonary mucoepidermoid carcinoma), OVCAR-3

30 (ovarian carcinoma), RD (rhabdosarcoma), THP (monocyte leukemia), WIL2-NS (B lymphocyte line, non-secreting), WISH (amnion).

Example 31:

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S-SPV-051

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S-SPV-051 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for the bovine viral diarrhea virus glycoprotein 53 (g53) were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the BVDV g53 gene is under the control of the synthetic late/early promoter (LP2EP2).

10

S-SPV-051 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 783-39.2 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 051. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

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S-SPV-051 was assayed for expression of BVDV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. A mouse monoclonal antibody to BVDV g53 was shown to react specifically with S-SPV-051 plaques and not with S-SPV-001 negative control plaques. All S-SPV-051 observed plaques reacted with the monoclonal antibody to BVDV g53 indicating that the virus was stably expressing the BVDV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4

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cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the BVDV g53 gene product, cells were infected with S-SPV-051 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A mouse monoclonal antibody to BVDV g53 was used to detect expression of BVDV specific proteins. The cell lysate and supernatant from S-SPV-051 infected cells exhibited bands at 53 kd and higher indicating glycosylated and unglycosylated forms of the BVDV g53 protein.

S-SPV-051 is useful as a vaccine in cattle against BVDV infection and is useful for expression of BVDV glycoprotein 53.

Example 32:

S-SPV-044:

S-SPV-044 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for the infectious bursal disease virus (IBDV) polymerase protein were inserted into the 617-48.1 ORF (a unique NotI site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the IBDV polymerase gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-044 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 749-75.78 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR

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GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-044. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-044 is useful for expression of IBDV polymerase protein. S-SPV-044 is useful in an in vitro approach to a recombinant IBDV attenuated vaccine. RNA strands from the attenuated IBDV strain are synthesized in a bacterial expression system using T3 or T7 promoters (pBlueScript plasmid; Stratagene, Inc.) to synthesize double stranded short and long segments of the IBDV genome. The IBDV double stranded RNA segments and S-SPV-044 are transfected into CEF cells. The swinepox virus expresses the IBDV polymerase but does not replicate in CEF cells. The IBDV polymerase produced from S-SPV-044 synthesizes infectious attenuated IBDV virus from the double stranded RNA genomic templates. The resulting attenuated IBDV virus is useful as a vaccine against infectious bursal disease in chickens.

Example 33:

S-SPV-046:

S-SPV-046 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for the feline immunodeficiency virus (FIV) gag protease (gag) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L

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ORF; Deletion of nucleotides 1669 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox O1L promoter, and the FIV gag gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-046 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 761-75.B18 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 046. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

To confirm the expression of the FIV gag gene product, cells were infected with S-SPV-046 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Feline anti-FIV (PPR strain) sera was used to detect expression of FIV specific proteins. The cell lysate and supernatant from S-SPV-046 infected cells exhibited bands at 26 kd and 17 kd which are the expected sizes of the processed form of the FIV gag protein. The recombinant swinepox virus expressed FIV gag protein is processed properly and secreted into the culture media.

S-SPV-048

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S-SPV-048 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for feline immunodeficiency virus (FIV) envelope (env) were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the FIV env gene is under the control of the synthetic late/early promoter (LP2EP2).

10

S-SPV-048 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 781-84.C11 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 048. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

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S-SPV-046 and S-SPV-048 are useful alone or in combination as a vaccine in cats against FIV infection and are useful for expression of the FIV env and gag proteins. A recombinant swinepox virus expressing both the FIV env and gag proteins is useful as a vaccine in cats against FIV infection.

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Recombinant swinepox virus expressing human respiratory syncytial virus F and G proteins is useful as a vaccine against the human disease.

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Example 34In Vitro Properties of Chicken IFN Expressed in Recombinant Pox viruses.

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Growth properties of recombinant viruses in cell culture. Growth properties of recombinant S-SPV-042 were not effected in embryonic swine kidney cells (ESK-4) compared to wild-type swinpx virus.

10

Western blot analysis was performed on supernatants from cells infected with SPV/cIFN recombinant virus. Rabbit and mouse antisera were raised against cIFN from concentrated SPV/cIFN infected supernatants and pre-cleared against ESK-4 cells infected with wild-type SPV in preparation for western analysis. Rabbit and mouse anti-cIFN antisera were reacted with denatured proteins on nitrocellulose from recombinant SPV/cIFN and SPV wild type virus infected supernatants. A reactive band with an estimated molecular weight size range of 17-20 kilodaltons was present in the SPV/cIFN lanes, and absent in the SPV wild type control lanes.

25

Effect of cIFN expressed in supernatants from SPV/cIFN (S-SPV-042), FPV/cIFN, and FPV/cIFN/NDV infected cells on the growth of Vesicular Stomatitis Virus.

30

Virion cleared supernatants from SPV/cIFN, FPV/cIFN and FPV/cIFN/NDV infected cells were tested for the presence of viral inhibitory activity, results shown in Table 1. Briefly, CEF cells were incubated with serially diluted viral supernatants. Subsequently, 40,000 plaque forming units (pfu)/well of vesicular stomatitis virus (VSV) were added and 48 hours later, wells were scored for the presence of VSV cytopathic effect (CPE). Recombinant viral supernatants

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containing cIFN were shown to inhibit VSV induced CPE, whereas, control viral supernatants did not. VSV induced cytopathic effect could be reversed in the presence of rabbit anti-cIFN sera.

5

Table 1.

	Recombinant Viral Supernatants.	cIFN Activity (units/ml). ^a
	SPV/IFN	2,500 000
10	SPV	<100
	FPV/IFN	250,000
	FPV/cIFN/NDV	250,000
	FPV	<100

15 ^a. One unit of cIFN activity is defined as the dilution of pox virus supernatant at which 100% VSV CPE was inhibited.

20 Effect of cIFN expressed from supernatants of SPV/cIFN infected cells on herpes virus of turkeys.

Supernatant containing recombinant cIFN from ESK-4 cells infected with SPV/cIFN virus, was tested for its
25 ability to inhibit the growth of herpes virus of turkeys (HVT) in CEF cells, results shown in Table 2. Briefly, serially diluted supernatants were incubated with CEF cells, and then subsequently infected with 100 pfu/well of wild-type HVT. Plaques were counted in
30 all wells after 48 hours. It was shown that 10-100 units of cIFN activity inhibited plaque formation of HVT(100 pfu/well). Supernatants from wild type SPV did not inhibit HVT plaque formation.

35

Table 2.

SPV/cIFN Supernatant (units/ml ^a)	Number of HVT plaques
0	99
1000	0
100	0
10	45

a. One unit of cIFN activity is defined as the dilution of pox virus supernatant at which 100% VSV CPE was inhibited.

Induction of NO by chicken macrophages after treatment with cIFN expressed in supernatants from SPV/cIFN infected cells.

HD 11 cells or bone marrow adherent cells were incubated with 1000unit/ml of cIFN from SPV/cIFN supernatants, lipopolysaccharide (LPS) (6ng/ml) or with both cIFN and LPS, results shown in Table 3. After 24 hours, supernatant fluids were collected and nitrite levels were measured. These data demonstrate that cIFN expressed from SPV/cIFN supernatants has the ability to activate chicken macrophages in the presence of LPS.

Table 3.

Nitrite (micro/mol) levels following stimulation with :			
Cell source	LPS	SPV/cIFN	LPS + SPV/cIFN
HD11	10.76	6.4	35.29
BMAC	13.1	5.8	35.10

Conclusions:

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1. Recombinant swinepox viruses express biologically active chicken interferon into the supernatants of infected cells, as measured by protection of CEF cells from VSV infection.

5

2. Chicken interferon expressed in supernatants from recombinant SPV/cIFN infected cells has been shown to protect CEF cells against infection with HVT in a dose dependent manner.

10

3. Chicken interferon expressed from SPV/cIFN acted synergistically with LPS to activate chicken macrophages as detected by nitric oxide induction.

15

4. The foregoing data indicate that recombinant swinepox viruses expressing chicken IFN may have beneficial applications as immune modulating agents in vitro, in vivo and in ovo.

20

Example 35

As an alternative to the construction of a IBD vaccine using a viral vectored delivery system and/or subunit approaches, IBD virus RNA is directly manipulated re-constructing the virus using full length RNA derived from cDNA clones representing both the large (segment A) and small (segment B) double-stranded RNA subunits. Generation of IBD virus in this manner offers several advantages over the first two approaches. First, if IBD virus is re-generated using RNA templates, one is able to manipulate the cloned cDNA copies of the viral genome prior to transcription (generation of RNA). Using this approach, it is possible to either attenuate a virulent IBD strain or replace the VP2 variable region of the attenuated vaccine backbone with that of virulent strains. In doing so, the present invention provides protection against the virulent IBDV strain

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while providing the safety and efficacy of the vaccine strain. Furthermore, using this approach, the present invention constructs and tests temperature sensitive IBD viruses generated using the RNA polymerase derived from the related birnavirus infectious pancreatic necrosis virus (IPNV) and the polyprotein derived from IBDV. The IPNV polymerase has optimum activity at a temperature lower than that of IBDV. If the IPNV polymerase recognizes the regulatory signals present on IBDV, the hybrid virus is expected to be attenuated at the elevated temperature present in chickens. Alternatively, it is possible to construct and test IBD viruses generated using the RNA polymerase derived from IBDV serotype 2 virus and the polyprotein derived from IBDVserotype 1 virus..

cDNA clones representing the complete genome of IBDV (double stranded RNA segments A and B) is constructed, initially using the BursaVac vaccine strain (Sterwin Labs). Once cDNA clones representing full length copies of segment A and B are constructed, template RNA is prepared. Since IBDV exists as a bisegmented double-stranded RNA virus, both the sense and anti-sense RNA strands of each segment are produced using the pBlueScript plasmid; Stratagene, Inc.). These vectors utilize the highly specific phage promoters SP6 or T7 to produce substrate amounts of RNA in vitro. A unique restriction endonuclease site is engineered into the 3' PCR primer to linearize the DNA for the generation of run-off transcripts during transcription.

The purified RNA transcripts (4 strands) are transfected into chick embryo fibroblasts (CEF) cells to determine whether the RNA is infectious. If IBD virus is generated, as determined by black plaque assays using IBDV specific Mabs, no further manipulations are required and engineering of the

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vaccine strain can commence. The advantage of this method is that engineered IBD viruses generated in this manner will be pure and require little/no purification, greatly decreasing the time required to generate new vaccines. If negative results are obtained using the purified RNA's, functional viral RNA polymerase is required by use of a helper virus. Birnaviruses replicate their nucleic acid by a strand displacement (semi-conservative) mechanism, with the RNA polymerase binding to the ends of the double-stranded RNA molecules forming circularized ring structures (Muller & Nitschke, Virology 159, 174-177, 1987). RNA polymerase open reading frame of about 878 amino acids in swinepox virus is expressed and this recombinant virus (S-SPV-044) is used to provide functional IBDV RNA polymerase in trans. Swinepox virus expressed immunologically recognizable foreign antigens in avian cells (CEF cells), where there are no signs of productive replication of the viral vector. In the present invention the IBDV polymerase protein is expressed in the same cells as the transfected RNA using the swinepox vector without contaminating the cells with SPV replication.

With the demonstration that IBD virus is generated in vitro using genomic RNA, an improved live attenuated virus vaccines against infectious bursal disease is developed. Using recombinant DNA technology along with the newly defined system of generating IBD virus, specific deletions within the viral genome, facilitating the construction of attenuated viruses are made. Using this technology, the region of IBDV responsible for virulence and generate attenuated, immunogenic IBDV vaccines are identified. The present invention provides a virulent IBD strain or replacement of the VP2 variable region of the attenuated vaccine backbone with that of a virulent strain, thus

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protecting against the virulent strain while providing the safety and efficacy of the vaccine strain.

5 Example 36

Effects of Rabbit anti-chicken interferon (cIFN) antibody on the growth of Herpes Virus of Turkeys.

10 Supernatants from SPV/cIFN (SPV 042) infected ESK-4 cells were harvested 48 hours after infection and then concentrated 5-10 times, by Centricon 10 columns (Amicon). One ml of concentrated supernatant was injected into a rabbit 3 times, at 3 week intervals,
15 and then bled. This rabbit antisera was then used in culture to study the effect of interferon on the growth of HVT. It was shown that anti-cIFN reverses the block to HVT (1:200) and VSV(1:80) growth induced by the addition of cIFN in plaque assays. Furthermore,
20 it was shown that the addition of anti-cIFN (1:100) in the media of CEFs transiently transfected with sub-plaqueing levels of HVT viral DNA, enhances the formation of HVT plaques (200 plaques/well). CEFs transfected with HVT DNA in the absence of anti-cIFN
25 did not yield plaques.

HVT is highly susceptible to interferon produced from CEFs and that when cIFN is blocked, HVT growth is enhanced.

30

Applications include: (1) Use antibody to cIFN as an additive to increase HVT titers in vaccine stocks; (2) Use antibody to cIFN as an additive to facilitate the formation of new recombinant HVT viruses via cosmid
35 reconstructions.

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S-SPV-063

S-SPV-063 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for swine influenza virus (SIV) NP (H1N1) were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the SIV NP gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-063 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 807-41.3 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 063. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-063 was assayed for expression of SIV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-SIV serum or a polyclonal goat anti-NP serum was shown to react specifically with S-SPV-063 plaques and not with S-SPV-001 negative control plaques. All S-SPV-063 observed plaques reacted with the swine anti-SIV serum or goat anti-NP serum indicating that the virus was stably expressing the SIV foreign gene. The assays

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described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

5

To confirm the expression of the SIV NP gene products, cells were infected with S-SPV-063 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis, 10 The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-SIV serum or a polyclonal goat anti-NP serum was used to detect expression of SIV specific proteins. The cell lysate and supernatant from S-SPV-063 infected cells exhibited 15 bands corresponding to 56 kd, which is the expected size of the SIV NP protein.

S-SPV-063 is useful as a vaccine in swine against SIV infection and is useful for expression of SIV NP. S- 20 SPV-063 is useful as a vaccine in combination with S-SPV-066 which expresses NA and S-SPV-065 which expresses SIV HA.

S-SPV-064

25

S-SPV-064 is a swinepox virus that expresses one foreign gene. The gene for E. coli β -glucuronidase (uidA) was inserted into the unique XhoI restriction site within the 6.9 kb SPV HindIII J genomic fragment. 30 The uidA gene is under the control of the synthetic late/early promoter (LP2EP2). The HindIII J genomic fragment contains part of the A50R ORF (aa 227 to 552). The unique XhoI site is not within the A50R ORF. The XhoI site is 25 kb from the 3'end of the swinepox virus 35 genome (62).

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S-SPV-064 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 807-42.28 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV.

- 5 Homology vector 807-42.28 was generated by insertion of a NotI fragment containing the LP2EP2 promoter uidA gene cassette from plasmid 551-47.23 (see Materials and Methods) into a NotI site (unique XhoI site converted to NotI by a DNA linker) in the SPV 6.9 kb HindIII J
10 fragment. The transfection stock was screened by the SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATICAL MARKER GENES. The final result of blue plaque purification is the recombinant virus designated S-SPV-064. This virus is assayed for β -glucuronidase
15 expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, plaques observed are blue indicating that the virus is pure, stable, and
20 expressing the foreign gene.

- S-SPV-064 is purified and expresses the foreign gene, E. coli uidA, indicating that the XhoI site within the 6.9 kb HindIII J fragment is a site non-essential for
25 virus growth and a stable insertion site for foreign genes. Recombinant swinepox virus utilizing this insertion site is useful for expression of foreign antigen genes, as a vaccine against disease or as an expression vector to raise antibodies to the expressed
30 foreign gene.

S-SPV-065

- S-SPV-065 is a swinepox virus that expresses at least
35 two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for swine influenza virus (SIV) HA (H1N1) were inserted into the SPV 617 48.1 ORF (a

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unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the SIV HA gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-065 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 807-84.8 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 065. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene. S-SPV-065 was assayed for expression of SIV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-SIV serum or a Polyclonal goat anti-HA serum was shown to react specifically with S-SPV-065 plaques and not with S-SPV-001 negative control plaques. All S-SPV-065 observed plaques reacted with the swine anti-SIV serum or the SIV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the SIV NP gene products, cells were infected with S-SPV-065 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis.

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The gel was blotted and analyzed using the WESTERN
BLOTTING PROCEDURE. A Polyclonal swine anti-SIV serum
or a Polyclonal goat anti-HA serum was used to detect
expression SIV specific proteins. The cell lysate and
5 supernatant from S-SPV-065 infected cells exhibited
bands corresponding to 64 kd, which is the expected
size of the SIV-HA protein.

S-SPV-065 is useful as a vaccine in swine against SIV
10 infection and is useful for expression of SIV HA. S-
SPV-065 is useful as a vaccine in combination with S-
SPV-066 which expresses NA and S-SPV-063 which
expresses SIV NP.

15 S-SPV-066

S-SPV-066 is a swinepox virus that expresses at least
two foreign genes. The gene for E. coli β -galactosidase
(lacZ) and the gene for swine influenza virus (SIV) NA
20 (H1N1) were inserted into the SPV 617--48.1 ORF (a
unique NotI restriction site has replaced a unique AccI
restriction site). The lacZ gene is under the control
of the synthetic late promoter (LP1), and the SIV NA
gene is under the control of the synthetic late/early
25 promoter (LP2EP2).

S-SPV-066 was derived from S-SPV-001 (Kasza Strain).
This was accomplished utilizing the homology vector
807-84.35 (see Materials and Methods) and virus S-SPV-
30 001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
GENERATING RECOMBINANT SPV. The transfection stock was
screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING
 β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final
result of red plaque purification was the recombinant
35 virus designated S-SPV 066. This virus was assayed for
 β -galactosidase expression, purity, and insert
stability by multiple passages monitored by the blue

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plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

5

To confirm the expression of the SIV NA gene products, cells were infected with S-SPV-066 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis.

10 The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A Polyclonal swine anti-SIV serum or a Polyclonal goat anti-NA serum was used to detect expression of SIV specific proteins. The cell lysate and supernatant from S-SPV-066 infected cells exhibited
15 bands corresponding to 64 kd, which is the expected size of the SIV HA protein.

S-SPV-066 is useful as a vaccine in swine against SIV infection and is useful for expression of SIV-NA. S-
20 SPV-066 is useful as a vaccine in combination with S-SPV-065 which expresses HA and S-SPV-063 which expresses SIV NP.

S-SPV-071

25

S-SPV-071 is a swinepox virus that expresses at least four foreign genes. The gene for E. coli β -galactosidase (lacZ) and the genes for swine influenza virus (SIV) HA (H1N1) and NA (H1N1) were inserted into
30 the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the SIV HA, and NA genes are under the control of the synthetic late/early promoter
35 (LP2EP2).

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S-SPV-071 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 817-86.35 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
5 GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 071. This virus was assayed for
10 β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus
15 was pure, stable, and expressing the foreign gene.

S-SPV-071 was assayed for expression of SIV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal goat anti-HA
20 serum was shown to react specifically with S-SPV-071 plaques and not with S-SPV-001 negative control plaques. All S-SPV-071 observed plaques reacted with the goat anti-HA serum indicating that the virus was stably expressing the SIV foreign gene. The assays
25 described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

30 To confirm the expression of the SIV HA and NA gene products, cells were infected with S-SPV-071 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed
35 using the WESTERN BLOTTING PROCEDURE. A Polyclonal swine anti-SIV serum or a Polyclonal goat anti-HA serum

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was used to detect expression of SIV specific proteins. The cell lysate and supernatant from S-SPV-071 infected cells exhibited bands corresponding to 64 kd and 52 kd, which is the expected size of the SIV HA and NA
5 protein.

S-SPV-071 is useful as a vaccine in swine against SIV infection and is useful for expression of SIV-HA and NA. S-SPV-071 is useful as a vaccine in combination
10 with S-SPV-063 which expresses SIV NP.

S-SPV-074

S-SPV-074 is a swinepox virus that expresses at least
15 four foreign genes. The gene for E. coli β -glucuronidase (uidA) and the genes for swine influenza virus (SIV) HA (H1N1) and NA (H1N1) were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The uidA
20 gene is under the control of the synthetic late/early promoter (LP2EP2), and the SIV HA and NA genes are under the control of the synthetic late/early promoter (LP2EP2).

25 S-SPV-074 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 817.14.2 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was
30 screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 074. This virus was assayed for β -galactosidase expression, purity, and insert
35 stability by multiple passages monitored by the blue

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plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

5

S-SPV-074 was assayed for expression of SIV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-SIV serum was shown to react specifically with S-SPV-10 074 plaques and not with S-SPV-001 negative control plaques. All S-SPV-074 observed plaques reacted with the goat anti-HA serum indicating that the virus was stably expressing the SIV foreign gene. The assays described here were carried out in ESK-4 cells, 15 indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-074 is useful as a vaccine in a swine against SIV 20 infection and is useful for expression of SIV HA and NA. S-SPV-074 is useful as a vaccine in combination with S-SPV-063 which expresses SIV NP. S-SPV-063, -065, -066, -071, and -074, are useful alone or in combination as a vaccine in swine against swine 25 influenza infection and are useful for expression of the SIV NP, HA, and NA proteins.

S-SPV-068:

30 S-SPV-068 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for chicken macrophage migration inhibitory factor (cMIF) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; 35 Deletion of nucleotides 1679 to 2452, SEQ ID NO: 189).

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The lacZ gene is under the control of the swinepox OIL promoter, and the cMIF gene is under the control of the synthetic late/early promoter (LP2EP2).

5 S-SPV-068 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 802-95.A1 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
10 GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-068. This virus was assayed for β -galactosidase expression, purity, and insert
15 stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

20 To confirm the expression of the cMIF gene product, cells were infected with S-SPV-068 and samples of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted
25 and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal goat anti-human cMIF antibody was used to detect expression of cMIF specific proteins. The cell lysate from S-SPV-068 infected cells exhibited a band corresponding to approximately 15 kd, which is the
30 expected size of the cMIF protein.

S-SPV-068 is useful as a vaccine in chickens to inhibit migration of macrophages and to stimulate an immune response against infection by avian pathogens. S-SPV-
35 068 is useful for expression of cMIF.

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HOMOLOGY VECTOR 802-95.A1. The plasmid 802-95.A1 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* β -galactosidase (lac Z) marker gene and an chicken macrophage migration inhibitory factor (cMIF) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a swinepox virus 01L gene promoter and the cMIF gene is under the control of the late/early promoter (LP2EP2). The LP2EP2 cMIF gene cassette was inserted into a BamHI site of homology vector 752-22.1. Homology vector 802-95.A1 was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5' GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair BamHI to PvuII fragment derived from plasmid pJF751 (49) containing the *E. coli* lacZ gene. Fragment 3 is an approximately 363 base pair BglII fragment coding for the cMIF gene (63) derived by reverse transcription and polymerase chain reaction

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(PCR) (Sambrook, et al., 1989) of RNA ISOLATED FROM CONCANAVALLIN A STIMULATED CHICKEN SPLEEN CELLS. The antisense primer used for reverse transcription and PCR was 5' TCGAAGATCTTCTCATGCAAAGGTGGAACCGTTC -3' (6/95.28; SEQ ID NO: 58). The sense primer used for PCR was 5' TCGAAGATCTCATGCCTATGTTCACCATCCACAC -3' (6/95.27; SEQ ID NO: 59). The DNA fragment contains the open reading frame of 121 amino acids of the chicken macrophage migration inhibitory factor protein. The native methionine codon of cMIF is preceded by amino acid codons for met-asn-ser-asp-lys. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' (and 5' GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

S-SPV-069

20 S-SPV-069 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for human respiratory syncytial virus (HRSV) fusion (F) protein were inserted into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV OIL ORF; Deletion of nucleotides 1669 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox P_{OIL} promoter and the HRSV F gene is under the control of the synthetic late/early promoter (LP2EP2).

25 S-SPV-069 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 810-29.A2 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was

35 screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING

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5 β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 069. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

10

S-SPV-069 was assayed for expression of HRSV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Monoclonal antibody 621 (Biodesign, Inc.) against HRSV F was shown to react specifically with S-SPV-069 plaques and not with S-SPV-001 negative control plaques. All S-SPV-069 observed plaques reacted with the monoclonal antibody 621 indicating that the virus was stably expressing the PRV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

25 S-SPV-078

25

S-SPV-078 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for human respiratory syncytial virus (HRSV) attachment (G) protein were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late/early promoter (LP2EP2), and the HRSV G gene is under the control of the synthetic late/early promoter (LP2EP2).

30

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S-SPV-078 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 822-52G.7 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
5 GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification is the recombinant virus designated S-SPV-078. This virus is assayed for
10 β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is
15 pure, stable, and expressing the foreign gene.

S-SPV-069 and S-SPV-078 are useful individually or in combination as a vaccine in swine against human respiratory syncytial virus infection and are useful
20 for expression of HRSV F and G genes.

HOMOLOGY VECTOR 810-29.A2. The plasmid 810-29.A2 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase
25 (lac Z) marker gene and a human respiratory syncytial virus (HRSV) fusion (F) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair
30 fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the
35 control of a swinepox virus 01L gene promoter and the

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HRSV F gene is under the control of the late/early promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5' GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair BamHI to PvuII fragment derived from plasmid pJF751 (49) containing the E. coli lacZ gene. Fragment 3 is an approximately 1728 base pair EcoRI restriction fragment synthesized by reverse transcriptase and polymerase chain reaction (PCR) (15, 42) using RNA from the HRSV Strain A2 (ATCC VR-1302). The primer (5' GCCGAATTCGCTAATCCTCAAAGCAAATGCAAT-3'; 4/95.23) synthesizes from the 5' end of the HRSV F gene, introduces an EcoRI site at the 5' end of the gene and an ATG start codon. The primer (5'-GGTGAATTCTTTATTAGTTACTAAATGCAATATTATTT-3'; 4/95.24) synthesizes from the 3' end of the HRSV F gene and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1728 base pairs in length corresponding to the HRSV F gene. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5'

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GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

HOMOLOGY VECTOR 822-52G.7. The plasmid 822-52G.7 was
5 constructed for the purpose of inserting foreign DNA
into SPV. It incorporates an *E. coli* β -galactosidase
(lacZ) marker gene and the human respiratory syncytial
virus (HRSV) attachment (G) gene flanked by SPV DNA.
Upstream of the foreign genes is an approximately 1484
10 base pair fragment of SPV DNA. Downstream of the
foreign genes is an approximately 2149 base pair
fragment of SPV DNA. When this plasmid is used
according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
GENERATING RECOMBINANT SPV a virus containing DNA
15 coding for the foreign genes will result. Note that
the β -galactosidase (lacZ) marker gene is under the
control of a synthetic late/early pox promoter (LP2EP2)
and the HRSV G gene is under the control of a synthetic
late/early pox promoter (LP2EP2). It was constructed
20 utilizing standard recombinant DNA techniques (22 and
30), by joining restriction fragments from the
following sources. The plasmid vector was derived from
an approximately 2972 base pair HindIII to BamHI
restriction fragment of pSP65 (Promega). Fragment 1 is
25 an approximately 1484 base pair AccI to BglII
restriction sub-fragment of the SPV HindIII fragment M
(23). Fragment 2 is an approximately 3006 base pair
BamHI to PvuII restriction fragment of plasmid pJF751
(11). Fragment 3 is an approximately 899 base pair
30 EcoRI restriction fragment synthesized by reverse
transcriptase and polymerase chain reaction (PCR) (15,
42) using RNA from the HRSV Strain A2 (ATCC VR-1302).
The primer (5' GCCGAATTCCAAAAACAAGGACCAACGCAC-
3'; 4/95.25) synthesizes from the 5' end of the HRSV F
35 gene, introduces an EcoRI site at the 5' end of the

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gene and an ATG start codon. The primer (5'-GCCGAATTCACTACTGGCGTGGTGTGTTG-3'; 4/95.26) synthesizes from the 3' end of the HRSV G gene and was used for reverse transcription and polymerase chain reaction.

5 The PCR product was digested with EcoRI to yield a fragment 899 base pairs in length corresponding to the HRSV G gene. Fragment 4 is an approximately 2149 base pair HindIII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23).

10

HOMOLOGY VECTOR 807-41.3. The plasmid 807-41.3 was used to insert foreign DNA into SPV. It incorporates an E. coli B-galactosidase (lacZ) marker gene and the swine influenza virus (SIV) nucleoprotein (NP) gene flanked

15 by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the B galactosidase (lacZ) marker gene is under the control of a synthetic

late pox promoter (LP1) and the SIV NP gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following

20 sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to Bam HI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base BglII to AccI restriction sub-fragment of the SPV HindIII fragment M(23). Fragment 2 is an approximately 1501 base pair EcoRI to EcoRI fragment of the SIV NP gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The primer

25 The plasmid vector was derived from an approximately 2972 base pair HindIII to Bam HI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base BglII to AccI restriction sub-fragment of the SPV HindIII fragment M(23). Fragment 2 is an approximately 1501 base pair EcoRI to EcoRI fragment of the SIV NP gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The primer

30 HindIII fragment M(23). Fragment 2 is an approximately 1501 base pair EcoRI to EcoRI fragment of the SIV NP gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The primer (5'CATGAATTCTCAAGGCACCAAACGATCATATGAAC-3'; 6/95.13) synthesizes from the 5' end of the SIV NP gene and introduces an EcoRI site at the 5' -

35 (5'CATGAATTCTCAAGGCACCAAACGATCATATGAAC-3'; 6/95.13)

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ATTTGAATTCAATTGTCATACTCCTCTCGCATTGTCT-3'; 6/95.14)

synthesizes from the 3' end of the SIV NP gene, introduces an EcoRI site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1501 base pairs in length corresponding to the SIV NP gene. Fragment 3 is approximately 3010 base pair BamHI to PuvII restriction fragment of plasmid pJF751 (11). Fragment 4 is approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV Hind III restriction fragment M (23)

HOMOLOGY VECTOR 807-84.8. The plasmid 807-84.8 was used to insert foreign DNA into SPV. It incorporates an E. coli B-galactosidase (lacZ) marker gene and the swine influenza virus (SIV) hemmagglutinin (HA) gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the B-galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the SIV HA gene is under the control of a synthetic late/early promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restricting fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M(23). Fragment 2 is an approximately 1721 base pair BamHI to BamHI fragment of the SIV HA gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The primer

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(5'CCGAGGATCCGGCAATACTATTAGTCTTGCTATGTACAT-3'; 6/95.5) synthesizes from the 5' end of the SIV HA gene and introduces an BamHI site at the 5' end of the gene. The primer (5'- CTCTGGATCCTAATTTAAATACATATTCTGCACTGTS-3'; 6/95.6) synthesizes from the 3' end of the SIV HA gene, introduces a Bam HI site at the 3' end of the gene, and was used for the reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1721 base pairs in length corresponding to the SIV HA gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to fragment M (23).

HOMOLOGY VECTOR 807-84.35. The plasmid 807-84.35 was used to insert foreign DNA into SPV. It incorporates an E. coli B-galactosidase (lacZ) marker gene and the swine influenza virus (SIV) neuraminidase (NA) gene flanked by SPV DNA. When this PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the B-galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the SIV NA gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30) by joining restricting fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 2 is an approximately 1414 base pair EcoRI to BglII fragment of the SIV NA gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The primer (5'

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AATGAATTCAAATCAAAAAATAATAACCATTGGGTCAAT-3'; 6.95.12) synthesizes from the 3' end of the SIV NA gene, introduces an EcoRI site at the 5' end of the gene. The primer (5'-GGAAGATCTACTTGTCAATGGTGAATGGCAGATCAG-3'; 6/95.13) synthesizes from the 3' end of the SIV NA gene, introduces an BglII site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1414 base pairs in length corresponding to the SIV NA gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23).

HOMOLOGY VECTOR 807-86.35. The plasmid 807-86.35 was used to insert foreign DNA into SPV. It incorporates an E. coli B-galactosidase (lacZ) marker gene and the swine influenza virus (SIV) HEMAGGLUTININ (HA) and neuraminidase (NA) gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the B-galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the SIV NA and HA genes are each under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23).

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Fragment 2 is an approximately 1721 base pair BamHI to BamHI fragment for the SIV HA gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The primer (5' - CCGAGGATCCGGCAATACTATTAGTCTTGCTATGTACAT-3'; 6/95.5) synthesizes from the 5' end of the SIV HA gene and introduces a BamHI site at the 5' end of the gene. The primer (5' - CTCTGGGATCCTAATTTTAAATACATATTCTGCACTGTA-3'; 6/95.6) synthesizes from the 3' end of the SIV HA gene, introduces a BamHI site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1721 base pairs in length corresponding to the SIV HA gene. Fragment 3 is an approximately 1414 base pair EcoRI to BglIII fragment of the SIV NA gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The primer (5' - AATGAATTCAAATCAAAAAATAAACCATTGGGTCAAT-3'; 6/95.12) synthesizes from the 5' end of the SIV NA gene and introduces an EcoRI site at the 5' end of the gene. The primer (5' - GGAAGATCTACTTGTCAATGGTGAATGGCAGATCAG-3'; 6/95.13) synthesizes from the 3' end of the SIV NA gene, introduces a BglIII site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1414 base pairs in length corresponding to the SIV NA gene. Fragment 4 is an approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23).

35

HOMOLOGY VECTOR 817-14.2. The plasmid 817-14.2 was used to insert foreign DNA into SPV. It incorporates an E.

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coli B-galactosidase (lacZ) marker gene and the swine influenza virus (SIV) HEMAGGLUTININ (HA) and neuraminidase (NA) gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the B-galactosidase (uidA) marker gene is under the control of a synthetic late/early pox promoter (LP2EP2) and the SIV NA and HA genes are each under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction subfragment to the SPV HindIII fragment M (23). Fragment 2 is an approximately 1721 base pair BamHI to BamHI fragment of the SIV HA gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The primer (5' CCGAGGATCCGGCAATACTATTAGTCTTGCTATGTACAT-3'; 6/95.5) synthesizes from the 5' end of the SIV HA gene and introduces an BamHI site at the 5' end of the gene. The primer (5'-CTCTGGGATCCTAATTTTAAATACATATTCTGCACTGTA-3'; 6/95.6) synthesizes from the 3' end of the SIV HA gene, introduces an BamHI site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1721 base pairs in length corresponding to the SIV HA gene. Fragment 3 is an approximately 1414 base pair EcoRI to BglII fragment of the SIV HA gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using

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RNA for the SIV H1N1 strain (NVSL). The primer (5' AATGAATTCAAATCAAAAAATAATAACATTGGGTCAAT-3'; 6/95.12) synthesizes from the 5' end of the SIV NA gene, introduces an EcoRI site at the 5' end of the gene. The
5 primer (5'-GGAAGATCTACTTGTCAATGGTGAATGGCAGATCAG-3'; 6/95.13) synthesizes from the 3' end of the SIV NA gene, introduces an BglII site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested
10 with EcoRI to yield a fragment 1414 base pairs in length corresponding to the SIV NA gene. Fragment 4 is an approximately 1823 base pair NotI restriction fragment of plasmid pRAJ260 (Clonetech). Fragment 5 is an approximately 2149 base pair AccI to HindIII
15 restriction sub-fragment of the SPV HindIII restriction fragment M (23).

PRRS HOMOLOGY VECTORS CONTAINING SINGLE OR MULTIPLE PRRS GENES (ORF2, ORF3, ORF4, ORF5, ORF6 or ORF7: The
20 PRRS homology vector is constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli B-galactosidase (lacZ) marker gene and a porcine reproductive and respiratory syndrome virus (PRRS) ORF2, ORF3, ORF4, ORF5, ORF6 or ORF7 gene flanked by
25 SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION
30 PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the B-galactosidase (lacZ) marker gene is under the control of a swinepox virus 01L gene promoter and the PRRS gene is under the control of the
35 late/early promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA sequences. The plasmid vector was derived from an

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approximately 2519 base pair HINDIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV polymerase chain reaction using DNA primers 5' GAAGCATGCCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair BamHI to PvuII fragment derived from plasmid pJF751 (49) containing the E. coli lacZ gene. Fragment 3 is an EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the NVSL (Reference strain). Each homology vector contains one or multiple of the PRRS virus ORF2 through 7. To synthesize PRRS ORF2, the primer (5' AATGAATTCGAAATGGGTCCATGCAAAGCCTTTTGTG-3'; 1/96.15) synthesizes from the 5' end of the PRRS ORF2 gene, introduces an EcoRI site at the 5' end of the gene. The primer (5'- CAAGGATCCCACACCGTGTAATTCAGTGTGAGTTCG-3'; 1/96.16) is used for reverse transcription and PCR and synthesizes from the 3' end of the PRRS ORF2 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment 771 base pairs in length corresponding to the PRRS ORF2 gene. To synthesize PRRS ORF3, the primer (5' TTCGAATTCGGCTAATAGCTGTACATTCCTCCATATTT-3'; 1/96.7) synthesizes from the 5' end of the PRRS ORF3 gene, introduces an EcoRI site at the 5' end of the gene. The primer (5'- GGGGATCCTATCGCCGTACGGCACTGAGGG-3'; 1/96.8) is used for reverse transcription and PCR and synthesizes from the 3' end of the PRRS ORF3 gene. To synthesize PRRS ORF4, the primer (5' CCGAATTCGGCTGCGTCCCTTCTTTTCCTCATGG-3'; 1/96.11) synthesizes from the 5' end of the PRRS ORF4 gene, introduces an EcoRI site at the 5'-CTGGATCCTTCAAATTGCCAACAGAATGGCAAAAAGAC-3'; 1/96.12) is used for reverse transcription and PCR and synthesizes

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from the 3' end of the PRRS ORF4 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment 537 base pairs in length corresponding to the PRRS ORF4 gene. To synthesize PRRS ORF5, the primer (5' TTGAATTCGTTGGAGAAATGCTTGACCGCGGGC-3'; 1/96.13) synthesizes from the 5' end of the PRRS ORF5 gene, introduces an EcoRI site at the 5' end of the gene. The primer (5'- GAAGGATCCTAAGGACGACCCCATTTGTTCCGCTG-3'; 1/96.14) is used for reverse transcription and PCR and synthesizes from the 3' end of the PRRS ORF5 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment 603 base pairs in length corresponding to the PRRS ORF5 gene. To synthesize PRRS ORF6, the primer (5' CGGGAATTCGGGGTCGTCCTTAGATGACTTCTGCC-3'; 1/96.17) synthesizes from the 5' end of the PRRS ORF6 gene, introduces an EcoRI site at the 5' end of the gene. The primer (5' - GCGGATCCTTGTTATGTGGCATATTTGACAAGGTTTAC-3'; 1/96.18) is used for reverse transcription and PCR and synthesizes from the 3' end of the PRRS ORF6 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment 525 base pairs in length corresponding to the PRRS ORF6 gene. To synthesize PRRS ORF7, the primer (5' GTCGAATTCGCCAAATAACAACGGCAAGCAGCAGAAG-3'; 1/96.19) synthesizes from the 3' end of the PRRS ORF7 gene. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'- CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5' GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAT-3' to produce and 1113 base pair fragment with SalI and HindIII ends.

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Recombinant swinepox virus expressing pseudorabies genes

5 S-SPV-076 is a swinepox virus that expresses at least three foreign genes. The gene for E. coli B-galactosidase (lacZ) and the genes for pseudorabies virus (PRV) gD and gI were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under
10 the control of the synthetic late promoter (LP1), and the PRV gD and gI genes are under the control of the synthetic late/early promoter (LP2EP2).

15 S-SPV-077 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for pseudorabies virus (PRV) gI were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the
20 control of the synthetic late promoter (LP1), and the PRV gI gene is under the control of the synthetic late/early promoter (LP2EP2).

25 S-SPV-079 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for pseudorabies virus (PRV) gI were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the
30 control of the synthetic late promoter (LP1), and the PRV gB gene is under the control of the synthetic late/early promoter (LP2EP2).

35 S-SPV-076, S-SPV-077, S-SPV-079 have been tested by BLACK PLAQUE ASSAY and WESTERN BLOT for expression of the PRV glycoproteins.

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S-SPV-076, S-SPV-077, and S-SPV-079 were derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing a homology vector and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock were screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-076, S-SPV-077, and S-SPV-079. The viruses were assayed for B-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-076, S-SPV-077, and S-SPV-079 are useful as a vaccine in swine against PRV infection and is useful for expression of PRV gD, gI or gB. S-SPV-071 is useful as a vaccine in combination with a recombinant swinepox virus which expresses PRV gC, such as S-SPV-011, S-SPV-012 or S-SPV-013.

25	143B	carcinoma* osteosarcoma*
	A431	epidermoid carcinoma*
	A549	lung carcinoma*
	Capan-1	liver carcinoma*
	CF500	foreskin fibroblasts
30	Chang Liver	liver
	Detroit	Downs'foreskin fibroblasts
	HEL-199	embryonic lung
	HeLa	cervical carcinoma*
	Hep-2	epidermal larynx carcinoma
35	HISM	intestinal smooth muscle

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	HNK	neonatal kidney
	MRC-5	embryonic lung
	NCI-H292	pulmonary mucoepidermoid
	OVCAR-3	ovarian carcinoma*
5	RD	rhabdosarcoma*
	THP	monocyte (leukemia)*
	WIL2-NS	B lymphocyte line, non-secreting
	WISH	amnion
10	PBL	peripheral blood lymphocytes

15

20

Example 38

25 Recombinant swinepox virus expressing PRRS genes ORF2, ORF3, ORF4, ORF5 and ORF6

30 S-SPV-080 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF2 were inserted into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, (SEQ ID NO: 189). The lacZ gene is under the control of the swinepox P_{01L} promoter and the PRRS ORF2 gene is
35 under the control of the synthetic late/early promoter (LP2EP2).

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S-SPV-081 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF3 were inserted
5 into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, (SEQ ID NO: 189). The lacZ gene is under the control of the swinepox P_{O1L} promoter and the PRRS ORF3 gene is under the control of the synthetic late/early promoter
10 (LP2EP2).

S-SPV-082 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for porcine reproductive and
15 respiratory syndrome virus (PRRS) ORF4 were inserted into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, (SEQ ID NO: 189). The lacZ gene is under the control of the swinepox P_{O1L} promoter and the PRRS ORF4 gene is
20 under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-083 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase
25 (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF5 were inserted into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, (SEQ ID NO: 189). The lacZ gene is under the control of
30 the swinepox P_{O1L} promoter and the PRRS ORF5 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-084 is a swinepox virus that expresses at least
35 two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF6 were inserted

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into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, (SEQ ID NO: 189). The lacZ gene is under the control of the swinepox P_{o1l} promoter and the PRRS ORF6 gene is
5 under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-085 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli B-galactosidase
10 (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF7 were inserted into the SPV 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, (SEQ ID NO: 189). The lacZ gene is under the control of
15 the swinepox P_{o1l} promoter and the PRRS ORF7 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-080, S-SPV-081, S-SPV-082, S-SPV-083, S-SPV-084,
20 S-SPV-085 were derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector Material and Methods (PRRS HOMOLOGY VECTORS) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was
25 screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-080, S-SPV-081, S-SPV-082, S-SPV-083, S-SPV-084, S-SPV-085. This virus was assayed
30 for B-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus
35 was pure, stable, and expressing the foreign gene.

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S-SPV-080, S-SPV-081, S-SPV-082, S-SPV-083, S-SPV-084, S-SPV-085 are useful individually or in combination as vaccines in swine against PRRS infection and are useful for expression of PRRS ORF2, ORF3, ORF4, ORF5, ORF6 and
5 ORF7.

Example 39

The following experiment was performed to determine the
10 ability of swinepox virus to infect human cells in culture and express a foreign DNA as lacZ.

S-SPV-003 was absorbed to the human cell lines listed in the Table below at an MOI=0.1 for 2 to 3 hours.
15 Cells were rinsed three times with PBS, growth media was added, and cells were incubated at 37°C for four days. Cells were harvested and a lysate prepared in 200 microliters of PBS by freeze/thaw three times. Cell debris was pelleted, and 10 microliters of
20 supernatant was assayed for -galactosidase activity by ONPG assay at 37°C for 1 1/2 hours. The table shows the results of infection of various human cell lines with S-SPV-003 and the relative levels of cytopathetic effect and expression of lacZ.

25 The results show that various human cell lines vary in the ability to take up S-SPV-003 and express lacZ. CPE was minimal in all cases and did not result in viral replication. One exception A549 cells which did show
30 some rounding of cells and lifting off the plate in one instance, and another instance of ten-fold increase in titer during passage suggesting limited viral replication. Several cell lines show significant lacZ activity with no cytopathetic effect.

35 Different pox promoters express lacZ from recombinant swinepox virus in a number of human cell lines. Six

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different swinepox viruses were constructed which expressed lacZ from EP1, LP1, LP2, EP1LP2, LP2EP2, or the SPV P01L promoter. Viruses were each used to infect A549, Chang liver, or 143B cells at 0.1 moi, and
5 cells were rinsed between 2 and 3 hours later and then incubated for 4 days at 37°C. Each cell line maintained a different hierarchy of promoter activity, which was reproducible in following experiments.

- 10 For example, the EP1, LP2EP2, and P01L promoters gave the most expression in 143B cells, while the LP2 was strongest in Chang liver cells, and the EP1LP2 in A549. In the Chang liver and A549 cells, expression from the P01L promoter was poorest, whereas in 143B, expression
15 from LP2 was poorest. Therefore, different human cell lines utilize pox promoters in dissimilar ways. This may reflect how far the swinepox virus can proceed along the replication pathway in different cell lines.
- 20 These early and late promoters exhibited lower or higher lacZ activity depending on the human cell type infected by the recombinant swinepox virus. By choosing different promoters for different target tissues, one is able to regulate the amounts of foreign
25 gene product delivered by the swinepox virus to target tissues.

Recombinant swinepox virus is useful as a vaccine for human infectious disease and to deliver therapeutic
30 agents to humans. Recombinant swinepox virus is useful as a vaccine against viral or bacterial infection in humans, and as a therapeutic for cancer or genetic disease to deliver antibodies, tumor antigens, cell surface ligands and receptors, immune modulating
35 molecules such as cytokines.

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S-SPV-003 Expression of lacZ in human cell lines
 Measurement of cytopathic effect and lacZ expression

	Cell Type	Cytopathetic Effect*	LacZ Expression**
5	A431 epidermoid carcinoma*	_____	_____
	A549 lung carcinoma*	++	+++
10	Capan-1 liver carcinoma*	_____	_____
	CF500 foreskin fibroblasts	+	+
	Chang Liver	+	+++
15			
20			
25	Detroit Down's foreskin fibroblasts	+/-	_____
	HEL-199 embryonic lung	+/-	+++
30	HEp-2 epidermal larynx carcinoma*	_____	_____
	HISM intestinal smooth muscle	+	+
	HNK neonatal kidney	_____	++
35	MRC-5 embryonic lung	+/-	+
	NCI-H292 pulmonary mucoepidermoid carcinoma*	_____	+++

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	OVCAR-3	_____	+++
	ovarian carcinoma*		
	RD	_____	+
	rhabdosarcoma*		
5	THP	_____	+
	monocyte (leukemia)*		
	WIL2-NS	_____	_____
	B lymphocyte line, non-secreting		
10	WISH	+/-	++
	amnion		
	HeLa	_____	+++
	PBL	_____	_____
15	peripheral blood lymphocytes		

* When human cells are infected with SPV, a cytopathic effect is sometimes seen. In most cell lines, this cytopathic effect is evidenced by a change in the appearance of the cells, with cells becoming thinner and more ragged along the edges; cells look stressed. This phenomenon was assessed as follows:

25 - indicates no difference between infected & uninfected cells;

+/- indicates that the monolayer is visibly different from uninfected, though most cells appear normal;

30 + indicates that the monolayer is obviously affected, with most cells looking stressed. It should be noted that in certain cell lines (HeLa, CF500, 143B), in which titers were obtained after serial passage, there was no evidence for replication of SPV, with one exception.

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A549 was given a ++ for cytopathic effect in one instance, when cells appeared to round up and come off the plate during infection, though this observation was not repeated. A549 also showed evidence in another case of a ten-fold increase in titer during passage, suggesting that it might support limited viral replication.

10 ** B-galactosidase activity in A_{260} units per cell
lysate from 1/20 of a 35 mm dish:
- No activity
+ 0.2-0.9 A_{260} unit
++ 0.9-1.6 A_{260} unit
+++ greater than 1.6 A_{260} units.

15

20

Example 41: BOVINE CONSTRUCTS AND VACCINES

S-SPV-112

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S-SPV-112 is a swinepox virus that expresses three foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for bovine respiratory syncytial virus (BRSV) attachment (G) were inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The gene for BRSV fusion (F) was inserted into the unique PstI restriction site (PstI linkers inserted into a

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unique AccI site in the SPV 01L open reading frame).
The lacZ gene is under the control of the synthetic
late promoter (LP1), the BRSV G and F genes are each
under the control of the synthetic late/early promoter
5 (LP2EP2).

S-SPV-112 was derived from S-SPV-001 (Kasza Strain).
This was accomplished utilizing the homology vector
848-02 (see Materials and Methods) and virus S-SPV-001
10 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
GENERATING RECOMBINANT SPV. The transfection stock was
screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING
 β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final
result of red plaque purification was the recombinant
15 virus designated S-SPV 112. This virus was assayed for
 β -galactosidase expression, purity, and insert
stability by multiple passages monitored by the blue
plaque assay as described in Materials and Methods.
After the initial three rounds of purification, all
20 plaques observed were blue indicating that the virus
was pure, stable, and expressing the foreign gene.

S-SPV-112 is useful as a vaccine in bovine against
disease caused by bovine respiratory syncytial virus.
25 The BRSV antigens are key to raising a protective
immune response in the animal. The recombinant viruses
are useful alone or in combination as an effective
vaccine. The swinepox virus is useful for cloning other
subtypes of BRSV to protect against rapidly evolving
30 variants in this disease. S-SPV-112 is also useful as
an expression vector for expressing BRSV antigens. Such
BRSV antigens are useful to identify antibodies
directed against the wild-type BRSV. The virus is also
useful as a source of antigens for the production of
35 monospecific polyclonal or monoclonal antibodies. Such

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antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR
5 PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 848-02. The plasmid 848-02 was constructed for the purpose of inserting foreign DNA
10 into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene, the bovine respiratory syncytial virus (BRSV) attachment (G) and fusion (F) genes flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA.
15 Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will
20 result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the BRSV F and G genes are under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard
25 recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment
30 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 1722 base pair Bam HI fragment generated by PCR which contains the coding sequence of the BRSV F gene.
35 Fragment 3 is an approximately 48 base pair AccI to

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NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 771 base pair Bam HI fragment generated by PCR which contains the coding sequence for the BRSV G gene. The BRSV F and G genes were synthesized by PCR as described in the CLONING OF BOVINE RESPIRATORY SYNCYTIAL VIRUS FUSION, NUCLEOCAPSID AND GLYCOPROTEIN GENES. Fragment 5 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 6 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 6 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 6.

20

Recombinant swinepox virus expressing BRSV F and G fusion protein

S-SPV-130:

25

S-SPV-130 is a swinepox virus that expresses three foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The genes for bovine respiratory syncytial virus (BRSV) attachment (G) and BRSV fusion (F) are inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to

30
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2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the BRSV F/G fusion gene is under the control of the synthetic late/early promoter (LP2EP2). The BRSV F/G fusion gene comprises approximately 1560 nucleotides of the F gene (520 amino acids including the amino terminus) fused in frame to approximately 580 nucleotides of the G gene (193 amino acids including the carboxy terminus).

10 S-SPV-130 is derived from S-SPV-001 (Kasza Strain). This is accomplished utilizing the HOMOLOGY VECTOR 807-75.41 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
15 GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The recombinant virus is isolated by red plaque purification. This virus is assayed for β -galactosidase
20 expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus was pure, stable, and
25 expressing the foreign gene.

S-SPV-130 is useful as a vaccine in bovine against disease caused by bovine respiratory syncytial virus. The BRSV F/G fusion protein is particularly effective
30 and key to raising a protective immune response in the animal. The BRSV F/G fusion protein contains the intact amino terminus of the F protein and the intact carboxy terminus of the G protein which includes the known immunogenic region of each protein. The BRSV F/G fusion
35 protein provides an improved immune response compared

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to expressing the BRSV F and G proteins separately or
expressing isolated epitopes of the BRSV F and G
proteins. The swinepox virus is useful for cloning
other subtypes of BRSV to protect against rapidly
5 evolving variants in this disease. Recombinant swinepox
virus is also useful as an expression vector for
expressing BRSV antigens. Such BRSV antigens are useful
to identify antibodies directed against the wild-type
BRSV. The virus is also useful as a source of antigens
10 for the production of monospecific polyclonal or
monoclonal antibodies. Such antibodies are useful in
the development of diagnostic tests specific for the
viral proteins. Monoclonal or polyclonal antibodies are
generated in mice utilizing these viruses according to
15 the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS
FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 807-75.41. The homology vector 807-
75.41 was constructed for the purpose of inserting
20 foreign DNA into SPV. It incorporates an E. coli β -
galactosidase (lacZ) marker gene, a fusion protein of
the bovine respiratory syncytial virus (BRSV)
attachment (G) and fusion (F) genes flanked by SPV DNA.
Upstream of the foreign genes is an approximately 1484
25 base pair fragment of SPV DNA. Downstream of the
foreign genes is an approximately 1560 base pair
fragment of SPV DNA. When the plasmid is used according
to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
GENERATING RECOMBINANT SPV, a virus containing DNA
30 coding for the foreign genes will result. Note that the
 β galactosidase (lacZ) marker gene is under the control
of a synthetic late pox promoter (LP1), the BRSV F/G
fusion gene is under the control of a synthetic
late/early pox promoter (LP2EP2). It was constructed
35 utilizing standard recombinant DNA techniques (22, 30),

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by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector is derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1560 base pair Bam HI fragment generated by PCR which contains the coding sequence of the BRSV F gene. The F gene coding region from the BRSV strain 375 (VR-1339) was cloned using the following primers: 5'-GCGGATCCGGCGCGCCGGATTTTCCTACATCTACACT-3' (5/96.26; SEQ ID NO 12) for cDNA priming and combined with 5'-CTAAAATTGAATTGTAAT-3' (1/95.19; SEQ ID NO 13:) for PCR. The DNA encodes 520 amino acids at the amino terminus of the BRSV F protein. Fragment 5 is an approximately 580 base pair AscI fragment generated by PCR which contains the coding sequence for the BRSV G gene. The G gene coding region from the BRSV strain 375 (VR-1339) was cloned using the following primers: 5' TTGGCGCGCCCTAGATCTGTGTAGTTGATTGATTG-3' (5/96.28; SEQ ID NO 14:) for cDNA priming and combined with 5' TACGGCGCGCCGGGAAATGCTAAAGCCAAGCCCACA-3' (5/96.27; SEQ ID NO 15:) for PCR. The DNA product encodes 193 amino acids (including a translation stop codon) of the carboxy terminus of the BRSV G protein. The BRSV F and G coding sequences are fused in the correct translational reading frame. Fragment 6 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI

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sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 6 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 6.

10 S-SPV-099

S-SPV-099 is a swinepox virus that expresses two foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for bovine viral diarrhea virus type 2 (BVDV-2) (strain 890) glycoprotein 53 (gp53) was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the BVDV-2 gp53 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-099 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 815-73.16A (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant

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virus designated S-SPV 099. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods.

5 After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-099 is useful as a vaccine in bovine against
10 disease caused by bovine viral diarrhea virus. The BVDV-2 gp53 antigen is key to raising a protective immune response in the animal. The recombinant virus is useful alone or in combination as an effective vaccine. S-SPV-099 is also useful as an expression vector for
15 expressing BVDV antigens. Such BVDV antigens are useful to identify antibodies directed against the wild-type BVDV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in
20 the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

25
HOMOLOGY VECTOR 815-73.16A. The homology vector 815-73.16A was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and bovine viral
30 diarrhea virus type 2 (BVDV-2) glycoprotein 53 (gp53) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is
35 used according to the HOMOLOGOUS RECOMBINATION

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PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the BVDV gp53 gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1113 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the BVDV gp53 gene. BVDV gp53 gene coding region was cloned by reverse transcription and PCR using RNA from BVDV type 2 (Strain 890) as an RNA template for reverse transcription and the following PCR using primers: 5'-TTCGGATCCTGCTCAGACAGTATTGTGTATGTTATCAAGAGC-3' (2/96.32; SEQ ID NO 16:) at the 3' end of the BVDV gp53 gene for reverse transcription and PCR combined with 5'-CCATGAATTCCTTCCCTGAATGCAAGGAGGGCTTC-3' (2/96.15; SEQ ID NO 17:) at the 5' end of the BVDV gp53 gene for PCR. The DNA encodes approximately 373 amino acids of the BVDV gp53 protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and

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3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment
5 (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

S-SPV-109

10

S-SPV-109 is a swinepox virus that expresses two foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L
15 open reading frame). The gene for infectious bovine rhinotracheitis virus (IBRV) glycoprotein D (gD) was inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545
20 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the IBRV gD gene is under the control of the synthetic late/early promoter
25 (LP2EP2).

S-SPV-109 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 835-57.5 (see Materials and Methods) and virus S-SPV-
30 001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant
35 virus designated S-SPV 109. This virus was assayed for

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β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all
5 plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-109 is useful as a vaccine in bovine against disease caused for infectious bovine rhinotracheitis
10 virus. The IBRV gD antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-109 is also useful as an expression vector for expressing IBRV antigens. Such
15 IBRV antigens are useful to identify antibodies directed against the wild-type IBRV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such
antibodies are useful in the development of diagnostic
20 tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

25

HOMOLOGY VECTOR 835-57.5. The homology vector 835-57.5 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the infectious
30 bovine rhinotracheitis virus (IBRV) glycoprotein D (gD) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is
35 used according to the HOMOLOGOUS RECOMBINATION

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PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the IBRV gD gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1320 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV gD gene. The IBRV gD gene coding region was cloned by PCR using the HindIII K fragment of the IBRV Cooper strain (pSY 524) as DNA template and the following PCR primers: 5'-
25 CGGGATCCTCACCCGGGCAGCGCGCTGTA-3' (4/96.12; SEQ ID NO 18:) at the 3' end of the IBRV gD gene and combined with 5'- CGGAATTCACAAGGGCCGACATTGGCC-3' (4/96.11; SEQ ID NO 19:) at the 5' end of the IBRV gD gene. The DNA encodes approximately 440 amino acids of the IBRV gD protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I

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sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

5

S-SPV-110

S-SPV-110 is a swinepox virus that expresses two foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for infectious bovine rhinotracheitis virus (IBRV) glycoprotein I (gI) was inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the IBRV gI gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-110 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 835-58.5 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 110. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods.

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After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

5 S-SPV-110 is useful as a vaccine in bovine against disease caused for infectious bovine rhinotracheitis virus. The IBRV gI antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination
10 as an effective vaccine. S-SPV-110 is also useful as an expression vector for expressing IBRV antigens. Such IBRV antigens are useful to identify antibodies directed against the wild-type IBRV. The virus is also useful as a source of antigens for the production of
15 monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR
20 PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 835-58.5. The homology vector 835-58.5 was constructed for the purpose of inserting foreign
25 DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the infectious bovine rhinotracheitis virus (IBRV) glycoprotein I (gI) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA.
30 Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will
35 result. Note that the β galactosidase (lacZ) marker

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gene is under the control of a synthetic late pox promoter (LP1), the IBRV gI gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1140 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV_gI gene. The IBRV gI gene coding region was cloned by PCR using the HindIII K fragment of the IBRV Cooper strain (pSY 524) as DNA template and the following PCR primers: 5'-ATCGGGATCCCGTTATTCTTCGCTGATGGTGG-3' (4/96.18; SEQ ID NO 20) at the 3' end of the IBRV gI gene and combined with 5'-ATCGGAATTCGCGGTGCCTGTTGCTCTGGATG-3' (4/96.17; SEQ ID NO 21) at the 5' end of the IBRV gI gene. The DNA encodes approximately 380 amino acids of the IBRV gI protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to

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2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

S-SPV-111

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S-SPV-111 is a swinepox virus that expresses two foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for infectious bovine rhinotracheitis virus (IBRV) glycoprotein B (gB) was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the IBRV gB gene is under the control of the synthetic late/early promoter (LP2EP2). The direction of transcription of the IBRV gB gene is opposite the direction of transcription of the lacZ gene and the SPV 01L gene.

S-SPV-111 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 847-15.1C (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 111. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods.

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After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

5 S-SPV-111 is useful as a vaccine in bovine against disease caused for infectious bovine rhinotracheitis virus. The IBRV gB antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination
10 as an effective vaccine. S-SPV-111 is also useful as an expression vector for expressing IBRV antigens. Such IBRV antigens are useful to identify antibodies directed against the wild-type IBRV. The virus is also useful as a source of antigens for the production of
15 monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR
20 PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 847-15.1C. The homology vector 847-15.1C was constructed for the purpose of inserting
25 foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the infectious bovine rhinotracheitis virus (IBRV) glycoprotein B (gB) gene flanked by SPV DNA. The direction of transcription of the IBRV gB gene is opposite the direction of transcription of the lacZ gene and the SPV O1L gene.
30 Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according
35 to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR

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GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the IBRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 2800 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV gB gene. The IBRV gB gene coding region was cloned by PCR using the HindIII A fragment of the IBRV Cooper strain (pSY 830-71) as DNA template and the following PCR primers: 5' - CTTCGGATCCTCATGCCCCCGACGTCGGCCATC-3' (4/96.15; SEQ ID NO 22) at the 3' end of the IBRV gB gene and combined with 5' - TCATGAATTCTGGCCGCTCGCGGCGGTGCTGAACGC-3' (4/96.10; SEQ ID NO 23) at the 5' end of the IBRV gB gene. The DNA encodes approximately 932 amino acids of the IBRV gB protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An

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approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

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S-SPV-113

10 S-SPV-113 is a swinepox virus that expresses two foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for infectious bovine rhinotracheitis virus (IBRV) glycoprotein C (gC) was
15 inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been
20 deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the IBRV gC gene is under the control of the synthetic late/early promoter (LP2EP2).

25 S-SPV-113 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 848-08 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was
30 screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 113. This virus was assayed for β -galactosidase expression, purity, and insert
35 stability by multiple passages monitored by the blue

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plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

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S-SPV-113 is useful as a vaccine in bovine against disease caused by infectious bovine rhinotracheitis virus. The IBRV gC antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-113 is also useful as an expression vector for expressing IBRV antigens. Such IBRV antigens are useful to identify antibodies directed against the wild-type IBRV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

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HOMOLOGY VECTOR 848-08. The homology vector 848-08 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the infectious bovine rhinotracheitis virus (IBRV) glycoprotein C (gC) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will

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result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the IBRV gC gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1563 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV gC gene. The IBRV gC gene coding region was cloned by PCR using the HindIII I fragment of the IBRV Cooper strain (pSY 830-71) as DNA template and the following PCR primers: 5'-CGGGATCCCTAGGGCGCGGAGCCGAGGGC-3' (4/96.14; SEQ ID NO 24) at the 3' end of the IBRV gC gene and combined with 5'-CGGAATTCAGGCCCGCTGGGGCGAGCGTGG-3' (4/96.13; SEQ ID NO 25) at the 5' end of the IBRV gC gene. The DNA encodes approximately 521 amino acids of the IBRV gC protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to

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2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

5 S-SPV-115

S-SPV-115 is a swinepox virus that expresses two foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for infectious bovine rhinotracheitis virus (IBRV) glycoprotein B (gB) was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the IBRV gB gene is under the control of the synthetic late/early promoter (LP2EP2). The direction of transcription of the IBRV gB gene is the same as the direction of transcription of the lacZ gene and the SPV 01L gene.

25 S-SPV-115 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 847-19.59 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 115. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue

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plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

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S-SPV-115 is useful as a vaccine in bovine against disease caused for infectious bovine rhinotracheitis virus. The IBRV gB antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-115 is also useful as an expression vector for expressing IBRV antigens. Such IBRV antigens are useful to identify antibodies directed against the wild-type IBRV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 847-19.59. The homology vector 847-19.59 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the infectious bovine rhinotracheitis virus (IBRV) glycoprotein B (gB) gene flanked by SPV DNA. The direction of transcription of the IBRV gB gene is the same as the direction of transcription of the lacZ gene and the SPV OIL gene. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according

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to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the IBRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 2800 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV gB gene. The IBRV gB gene coding region was cloned by PCR using the HindIII A fragment of the IBRV Cooper strain (pSY 830-71) as DNA template and the following PCR primers: 5' - CTTCCGATCCTCATGCCCCCGACGTCGGCCATC-3' (4/96.15; SEQ ID NO 26) at the 3' end of the IBRV gB gene and combined with 5' - TCATGAATTCGGCCGCTCGCGCGGTGCTGAACGC-3' (4/96.10; SEQ ID NO 27) at the 5' end of the IBRV gB gene. The DNA encodes approximately 932 amino acids of the IBRV gB protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were

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converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span
5 SPV fragments 3 and 5.

S-SPV-119

10 S-SPV-119 is a swinepox virus that expresses three foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The genes for infectious bovine
15 rhinotracheitis virus (IBRV) glycoprotein D (gD) and glycoprotein I (gI) were inserted into the unique HindIII restriction site (HindIII linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment
20 (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the IBRV gD and gI genes are each under the control of the synthetic late/early promoter (LP2EP2).

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S-SPV-119 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 835-83 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
30 GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 119. This virus was assayed for
35 β -galactosidase expression, purity, and insert

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stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus was
5 pure, stable, and expressing the foreign gene.

S-SPV-119 is useful as a vaccine in bovine against disease caused for infectious bovine rhinotracheitis virus. The IBRV gD and gI antigens are key to raising
10 a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-119 is also useful as an expression vector for expressing IBRV antigens. Such IBRV antigens are useful to identify antibodies
15 directed against the wild-type IBRV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or
20 polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

25 **HOMOLOGY VECTOR 835-83.** The homology vector 835-83 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the infectious bovine rhinotracheitis virus (IBRV) glycoprotein D (gD) and
30 glycoprotein I (gI) genes flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS
35 RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV,

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a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the IBRV gI gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1320 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV gD gene. The IBRV gD gene coding region was cloned by PCR using the HindIII K fragment of the IBRV Cooper strain (pSY 524) as DNA template and the following PCR primers: 5'-CGGGATCCTCACCCGGGCAGCGCTGTA-3' (4/96.12; SEQ ID NO 18) at the 3' end of the IBRV gD gene and combined with 5'-CGGAATTCACAAGGGCCGACATTGGCC-3' (4/96.11; SEQ ID NO 19) at the 5' end of the IBRV gD gene. The DNA encodes approximately 440 amino acids of the IBRV gD protein. Fragment 5 is an approximately 1140 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the IBRV gI gene. The IBRV gI gene coding region was cloned by PCR using the HindIII K fragment of the IBRV Cooper strain (pSY 524) as DNA template and the following PCR primers: 5'-

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ATCGGGATCCCGTTATTCTTCGCTGATGGTGG-3' (4/96.18; SEQ ID NO
20) at the 3' end of the IBRV gI gene and combined with
5'-ATCGGAATTCGCGGTGCCTGTTGCTCTGGATG-3' (4/96.17; SEQ ID
NO 21) at the 5' end of the IBRV gI gene. The DNA
5 encodes approximately 380 amino acids of the IBRV gI
protein. Fragment 6 is an approximately 1560 base pair
NdeI to HindIII subfragment of the SPV HindIII fragment
M. The AccI sites in fragments 1 and 3 were converted
to unique PstI sites using PstI linkers. The NdeI sites
10 in fragments 3 and 6 were converted to unique Not I
sites using NotI linkers. An approximately 545 base
pair NdeI to NdeI subfragment (Nucleotides 1560 to
2104; SEQ ID NO. 189) of the SPV HindIII M fragment has
been deleted which would span SPV fragments 3 and 6.

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Example 42: CANINE CONSTRUCTS AND VACCINESS-SPV-114

5 S-SPV-114 is a swinepox virus that expresses two foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for canine parvovirus
10 (CPV) VP2 protein was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV
15 HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the CPV VP2 gene is under the control of the synthetic late/early promoter (LP2EP2). The direction of transcription of the CPV VP2 gene is the same as the
20 direction of transcription of the lacZ gene and the SPV 01L gene.

S-SPV-114 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector
25 848-15.14 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final
30 result of red plaque purification was the recombinant virus designated S-SPV 114. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods.
35 After the initial three rounds of purification, all

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plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

5 S-SPV-114 was assayed for expression of CPV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Canine antiserum to CPV (from NVSL) was shown to react specifically with S-SPV-114 plaques and not with S-SPV-003 negative control plaques. All S-SPV-114 observed plaques reacted with
10 the antiserum indicating that the virus was stably expressing the CPV foreign gene.

To confirm the expression of the CPV VP2 gene product, cells were infected with S-SPV-114 and samples of
15 infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A canine antiserum to CPV (from NVSL) was used to detect expression of CPV specific proteins. The cell lysate
20 from S-SPV-114 infected cells exhibited bands corresponding to 60 kd, which are the expected size of the CPV VP2 protein.

S-SPV-114 is useful as a vaccine in canine against
25 disease caused by canine parvovirus. The CPV VP2 antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-114 is also useful as an expression vector for expressing CPV
30 antigens. Such CPV antigens are useful to identify antibodies directed against the wild-type CPV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the
35 development of diagnostic tests specific for the viral

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proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

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HOMOLOGY VECTOR 848-15.14. The homology vector 848-15.14 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and for canine parvovirus (CPV) VP2 protein gene flanked by SPV DNA. The direction of transcription of the CPV VP2 gene is the same as the direction of transcription of the lacZ gene and the SPV O1L gene. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the CPV VP2 gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII

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M fragment. Fragment 4 is an approximately 1758 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the CPV VP2 gene. The CPV VP2 gene coding region was cloned by PCR using DNA
5 from CPV 2B field isolate (NVSL) as DNA template and the following PCR primers: 5'-
CGGGATCCTTAATATAATTTCTAGGTGCTAGTTG -3' (4/96.26; SEQ
ID NO 28) at the 3' end of the CPV VP2 gene and combined with 5'- CGGAATTCGATGAGTGATGGAGCAGTTCAA -3'
10 (4/96.25; SEQ ID NO 29) at the 5' end of the CPV VP2 gene. The DNA encodes approximately 586 amino acids of the CPV VP2 protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and
15 3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV
20 HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

S-SPV-116

25 S-SPV-116 is a swinepox virus that expresses two foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for canine parvovirus
30 (CPV) VP2 protein was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV
35 HindIII M fragment has been deleted). The lacZ gene is

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under the control of the synthetic late promoter (LP1), the CPV VP2 gene is under the control of the synthetic late/early promoter (LP2EP2). The direction of transcription of the CPV VP2 gene is opposite the
5 direction of transcription of the lacZ gene and the SPV OIL gene.

S-SPV-116 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector
10 848-15.13 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final
15 result of red plaque purification was the recombinant virus designated S-SPV 116. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods.
20 After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-116 was assayed for expression of CPV-specific
25 antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Canine antiserum to CPV (from NVSL) was shown to react specifically with S-SPV-116 plaques and not with S-SPV-003 negative control plaques. All S-SPV-116 observed plaques reacted with
30 the antiserum indicating that the virus was stably expressing the CPV foreign gene.

To confirm the expression of the CPV VP2 gene product, cells were infected with S-SPV-116 and samples of
35 infected cell lysates were subjected to SDS

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polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A canine antiserum to CPV (from NVSL) was used to detect expression of CPV specific proteins. The cell lysate
5 from S-SPV-116 infected cells exhibited bands corresponding to 60 kd, which are the expected size of the CPV VP2 protein.

S-SPV-116 is useful as a vaccine in canine against
10 disease caused by canine parvovirus. The CPV VP2 antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-116 is also useful as an expression vector for expressing CPV
15 antigens. Such CPV antigens are useful to identify antibodies directed against the wild-type CPV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the
20 development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

25

HOMOLOGY VECTOR 848-15.13. The homology vector 848-15.13 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and for canine
30 parvovirus (CPV) VP2 protein gene flanked by SPV DNA. The direction of transcription of the CPV VP2 gene is opposite the direction of transcription of the lacZ gene and the SPV O1L gene. Upstream of the foreign genes is an approximately 1484 base pair fragment of
35 SPV DNA. Downstream of the foreign genes is an

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approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes

5 will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the CPV VP2 gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard

10 recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment

15 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48

20 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 1758 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the CPV VP2 gene. The CPV VP2 gene coding region was cloned by PCR using DNA

25 from CPV 2B field isolate (NVSL) as DNA template and the following PCR primers: 5'-
CGGGATCCTTAATATAATTTTCTAGGTGCTAGTTG -3' (4/96.26; SEQ
ID NO 30) at the 3' end of the CPV VP2 gene and combined with 5'- CGGAATTCGATGAGTGATGGAGCAGTTCAA -3'

30 (4/96.25; SEQ ID NO 31) at the 5' end of the CPV VP2 gene. The DNA encodes approximately 586 amino acids of the CPV VP2 protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and

35 3 were converted to unique PstI sites using PstI

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linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

S-SPV-117

10 S-SPV-117 is a swinepox virus that expresses two foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV O1L open reading frame). The gene for canine parvovirus
15 (CPV) VP1/2 protein was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV
20 HindIII M fragment has been deleted. The lacZ gene is under the control of the synthetic late promoter (LP1), the CPV VP1/2 gene is under the control of the synthetic late/early promoter (LP2EP2). The direction of transcription of the CPV VP1/2 gene is opposite the
25 direction of transcription of the lacZ gene and the SPV O1L gene.

S-SPV-117 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector
30 848-52A31 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final
35 result of red plaque purification was the recombinant

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virus designated S-SPV 117. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods.

5 After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-117 is useful as a vaccine in canine against
10 disease caused by canine parvovirus. The CPV VP1/2 antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-117 is also useful as an expression vector for expressing CPV
15 antigens. Such CPV antigens are useful to identify antibodies directed against the wild-type CPV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the
20 development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

25

HOMOLOGY VECTOR 848-52A31. The homology vector 848-52A31 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and for canine
30 parvovirus (CPV) VP1/2 protein gene flanked by SPV DNA. The direction of transcription of the CPV VP1/2 gene is opposite the direction of transcription of the lacZ gene and the SPV O1L gene. Upstream of the foreign genes is an approximately 1484 base pair fragment of
35 SPV DNA. Downstream of the foreign genes is an

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approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the CPV VP1/2 gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 2172 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the CPV VP1/2 gene. The CPV VP1/2 gene coding region was cloned by PCR using DNA from CPV 2B field isolate (NVSL) as DNA template and the following PCR primers: 5'-CGGGATCCTTAATATAATTTTCTAGGTGCTAGTTG -3' (4/96.26; SEQ ID NO 32) at the 3' end of the CPV VP1/2 gene and combined with 5'-CGGAATTCTATGTGTTTTTTTATAGGACTT -3' (5/96.25; SEQ ID NO 33) at the 5' end of the CPV VP1/2 gene. The DNA encodes approximately 724 amino acids of the CPV VP1/2 protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique PstI sites using PstI

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linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

S-SPV-118

10

S-SPV-118 is a swinepox virus that expresses two foreign genes. The gene E. coli β -galactosidase (lacZ) is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV OlL open reading frame). The gene for canine parvovirus (CPV) VP1/2 protein was inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV OlL open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the CPV VP1/2 gene is under the control of the synthetic late/early promoter (LP2EP2). The direction of transcription of the CPV VP1/2 gene is the same as the direction of transcription of the lacZ gene and the SPV OlL gene.

S-SPV-118 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 848-52C8 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final

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result of red plaque purification was the recombinant virus designated S-SPV 118. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign gene.

10 S-SPV-118 is useful as a vaccine in canine against disease caused by canine parvovirus. The CPV VP1/2 antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-118 is also useful as an expression vector for expressing CPV antigens. Such CPV antigens are useful to identify antibodies directed against the wild-type CPV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 848-52C8. The homology vector 848-52C8 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and for canine parvovirus (CPV) VP1/2 protein gene flanked by SPV DNA. The direction of transcription of the CPV VP1/2 gene is the same as the direction of transcription of the lacZ gene and the SPV O1L gene. Upstream of the foreign genes is an approximately 1484 base pair fragment of

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SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the CPV VP1/2 gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 2172 base pair EcoRI/BamHI fragment generated by PCR which contains the coding sequence of the CPV VP1/2 gene. The CPV VP1/2 gene coding region was cloned by PCR using DNA from CPV 2B field isolate (NVSL) as DNA template and the following PCR primers: 5'-CGGGATCCTTAATATAATTTTCTAGGTGCTAGTTG -3' (4/96.26; SEQ ID NO 34) at the 3' end of the CPV VP1/2 gene and combined with 5'-CGGAATTCTATGTGTTTTTTTATAGGACTT -3' (5/96.25; SEQ ID NO 35) at the 5' end of the CPV VP1/2 gene. The DNA encodes approximately 724 amino acids of the CPV VP1/2 protein. Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and

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3 were converted to unique PstI sites using PstI linkers. The NdeI sites in fragments 3 and 5 were converted to unique Not I sites using NotI linkers. An approximately 545 base pair NdeI to NdeI subfragment 5 (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 3 and 5.

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Example 43: AVIAN CONSTRUCTS AND VACCINESS-SPV-105:

5 S-SPV-105 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for chicken interferon gamma (cIFN γ) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of
10 nucleotides 1679 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox O1L promoter, and the cIFN γ gene is under the control of the synthetic late/early promoter (LP2EP2).

15 S-SPV-105 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 840-72.A1 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was
20 screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-105. This virus was assayed for β -galactosidase expression, purity, and insert
25 stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

30 S-SPV-105 is confirmed to have cIFN γ activity by measuring the inhibition of vesicular stomatitis virus growth in permissive cells by cocultivation with S-SPV-105 compared to cocultivation of VSV with S-SPV-003.

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S-SPV-105 is useful as a vaccine in chickens to stimulate a humoral and cell mediated immune response against infection by avian pathogens. S-SPV-105 is useful for expression of cIFN γ .

5
HOMOLOGY VECTOR 840-72.A1. The plasmid 840-72.A1 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lac Z) marker gene and an chicken interferon gamma
10 (cIFN γ) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS
15 RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a swinepox virus 01L gene promoter and the cIFN γ gene is under the
20 control of the late/early promoter (LP2EP2). The LP2EP2 cIFN γ gene cassette was inserted into a EcoRI and BamHI site of homology vector 752-22.1. Homology vector 840-72.A1 was constructed utilizing standard recombinant DNA techniques (22, 30), by joining
25 restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the
30 SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5' GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3 and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends.
35 Fragment 2 is a 3002 base pair BamHI to PvuII fragment

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derived from plasmid pJF751 (49) containing the *E. coli* lacZ gene. Fragment 3 is an approximately 522 base pair EcoRI to BglII fragment coding for the cIFN γ gene (62) derived by reverse transcription and polymerase chain reaction (PCR) (Sambrook, et al., 1989) of RNA ISOLATED FROM CONCANAVALLIN A STIMULATED CHICKEN SPLEEN CELLS. The antisense primer used for reverse transcription and PCR was 5' CGTCAGATCTCAGGAGGTCATAAGATGCCATTAGC-3' (1/96.38; SEQ ID NO 36). The sense primer used for PCR was 5' CGTTGAATTCGATGACTTGCCAGACTTACAACCTTG-3' (1/96.37; SEQ ID NO 37). The DNA fragment contains the open reading frame of 168 amino acids of the chicken interferon gamma protein. The native methionine codon of cIFN γ is preceded by DNA codons for methionine-asparagine-serine. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5'-GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

S-SPV-086

S-SPV-086 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and antisense of the gene for chicken interferon gamma (cIFN γ) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV OIL ORF; Deletion of nucleotides 1679 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox OIL promoter, and the antisense-cIFN γ gene is under the control of the synthetic late/early promoter (LP2EP2).

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S-SPV-086 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 836-62.B1 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV.

5 The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-086. This virus was assayed for B-galactosidase

10 expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and

15 expressing the foreign gene. Homology vector 836-62.B1 is constructed in the same manner as homology vector 840-72.A1 except that in 836-62.B1, the approximately 522 base pair EcoRI to BgIII fragment coding for the cIFN γ gene is in the opposite orientation relative to

20 the LP2EP2 promoter compared to 840-72.A1.

S-SPV-086 was assayed for expression of B-galactosidase antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Anti-B-galactosidase

25 antiserum was shown to react specifically with S-SPV-086 plaques and not with S-SPV-003 negative control plaques. All S-SPV-086 observed plaques reacted with the antiserum indicating that the virus was stably expressing the B-galactosidase foreign gene.

30 S-SPV-086 is useful for expression of antisense RNA to the cIFN γ mRNA. When S-SPV-986 is transfected into duck embryo fibroblasts (DEF) or chicken embryo fibroblast (CEF) cells, it will not lyse the cells, but will

35 express antisense cIFN γ RNA in CEF cells and inhibit expression of cIFN γ protein from DEF or CEF cells. Recombinant viruses, such as herpesvirus of turkeys

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(HVT) or Marek's disease virus, grow to higher titers (10^8 to 10^{10} pfu/ml) in S-SPV-086 transfected into DEF or CEF cells, and transfected cells are selected for puromycin resistance by growth in the presence of puromycin. These transfected cells will grow continuously, express antisense to cIFN γ gene and permit high titer growth of herpesvirus to turkeys or Marek's disease virus (10^8 to 10^{10} pfu/ml).

10 Example 44: FELINE CONSTRUCTS AND VACCINES

S-SPV-106

S-SPV-106 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the genes for feline immunodeficiency virus (FIV) envelope (env) and gag-protease were inserted into the SPV 617 48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the FIV env and gag-protease genes are each under the control of the synthetic late/early promoter (LP2EP2).

25 S-SPV-106 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 836-22.A1 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 106. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue

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plaque assay as described in Materials and Methods. After the initial five rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

5

S-SPV-106 was assayed for expression of FIV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Monoclonal antibodies to FIV gag-protease were shown to react specifically with
10 S-SPV-106 plaques and not with S-SPV-003 negative control plaques. All S-SPV-106 observed plaques reacted with the antiserum indicating that the virus was stably expressing the FIV gag foreign gene.

15 S-SPV-106 is a recombinant swinepox virus expressing both the FIV env and gag-protease proteins and is useful as a vaccine in cats against FIV infection S-SPV-106 is also useful for expression of the FIV env and gag-protease proteins.

20

HOMOLOGY VECTOR 836-22.A1. The plasmid 836-22.A1 was used to insert foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the feline immunodeficiency virus (FIV) envelope (env) and
25 gag/protease genes flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β galactosidase (lacZ) marker
30 gene is under the control of a synthetic late pox promoter (LP1) and the FIV env and gag/protease genes are under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and
35 30), by joining restriction fragments from the

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following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 2 is an approximately 2564 base pair BamHI to BamHI fragment of the FIV env gene (61) (approximately 860 amino acids which includes the full length SU and TM coding regions of FIV env) synthesized by CLONING WITH THE POLYMERASE CHAIN REACTION. The template for the PCR reaction was FIV strain PPR genomic cDNA (61). The upstream primer 10/93.21 (5'-GCCCGGATCCTATGGCAGAAGGGTTGCAGC-3';) was synthesized corresponding to the 5' end of the FIV env gene starting at nucleotide 6263 of FIV strain PPR genomic cDNA, and the procedure introduced a BamHI site at the 5' end. The downstream primer 10/93.20 (5'-CCGTGGATCCGGCACTCCATCATTCCTCCTC-3';) was synthesized corresponding to the 3' end of the FIV env gene starting at nucleotide 8827 of FIV PPR genomic cDNA. Fragment 3 is an approximately 1878 base pair EcoRI to BglII restriction fragment of the FIV gag/protease (gag ORF is approximately 452 amino acids; protease ORF is approximately 209 amino acids) synthesized by polymerase chain reaction (PCR) using cDNA from the FIV (PPR strain) (61). The primer (5'-GCGTGAATTCTGGGGAATGGACAGGGGCGAGAT-3'; 11/94.9) synthesizes from the 5' end of the FIV gag-protease gene, introduces an EcoRI site at the 5' end of the gene. The primer (5'-GAGCCAGATCTGCTCTTTTACTTTCCC-3'; 11/94.10) synthesizes from the 3' end of the FIV gag-protease gene and introduces a BglII site at the 3' end of the gene. The PCR product was digested with EcoRI and BglII to yield a fragment 1878 base pairs in length

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corresponding to the FIV gag-protease gene. Fragment 4 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 5 is an approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology vector was converted to a unique NotI site.

S-SPV-127

10

S-SPV-127 is a swinepox virus that expresses four foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for the feline immunodeficiency virus (FIV) gag/protease (gag) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, SEQ ID NO: 189). The gene for E. coli β -glucuronidase (uidA) and the gene for the feline immunodeficiency virus (FIV) envelope (env) were inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region (SEQ ID NO 1) of the 6.7 kb SPV HindIII K fragment). The lacZ gene is under the control of the swinepox O1L promoter, the uidA gene is under the control of the synthetic early promoter (EP2) and the FIV gag/protease and envelope genes are each under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-127 was derived from S-SPV-046 (Kasza Strain). This was accomplished utilizing the homology vector 849-61.A14 (see Materials and Methods) and virus S-SPV-046 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase and β -glucuronidase (BLUOGAL AND CPRG

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ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of red plaque and blue plaque purification was the recombinant virus designated S-SPV-127. This virus was
5 assayed for β -galactosidase and β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are
10 blue indicating that the virus is pure, stable, and expressing the foreign genes.

S-SPV-127 is useful as a vaccine in cats against FIV infection S-SPV-127 is also useful for expression of
15 the FIV env and gag proteins.

HOMOLOGY VECTOR 849-61.A14. The plasmid 849-61.A14 was used to insert foreign DNA into SPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene and the
20 feline immunodeficiency virus (FIV) envelope (env) gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β -
25 glucuronidase (uidA) gene is under the control of a synthetic early pox promoter (EP2) and the FIV env gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and
30 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 3005 base pair HindIII restriction fragment of pSP65 (Promega). Fragment 1 is an
35 approximately 1652 base pair HindIII to EcoRI

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restriction sub-fragment of the SPV *HindIII* restriction
fragment K. Fragment 2 is an approximately 2564 base
pair *BamHI* to *BamHI* fragment of the FIV *env* gene (61)
(approximately 860 amino acids which includes the full
length SU and TM coding regions of FIV *env*) synthesized
by CLONING WITH THE POLYMERASE CHAIN REACTION. The
template for the PCR reaction was FIV strain PPR
genomic cDNA (61). The upstream primer 10/93.21 (5'-
GCCCGGATCCTATGGCAGAAGGGTTTGCAGC-3';) was synthesized
corresponding to the 5' end of the FIV *env* gene
starting at nucleotide 6263 of FIV strain PPR genomic
cDNA, and the procedure introduced a *BamHI* site at the
5' end. The downstream primer 10/93.20 (5'-
CCGTGGATCCGGCACTCCATCATTCCTCCTC-3';) was synthesized
corresponding to the 3' end of the FIV *env* gene
starting at nucleotide 8827 of FIV PPR genomic cDNA,
and the procedure introduced a *BamHI* site at the 3'
end. Fragment 3 is an approximately 1800 base pair
EcoRI to *XmaI* restriction fragment containing the *E.*
coli uidA gene. Fragment 4 is an approximately 3010
base pair *BamHI* to *PvuII* restriction fragment of
plasmid pJF751 (11). Fragment 5 is an approximately
5053 base pair *EcoRI* to *HindIII* restriction sub-
fragment of the SPV *HindIII* restriction fragment K. The
EcoRI site in fragments 1 and 5 of the SPV homology
vector was converted to a unique *NotI* site.

S-SPV-089

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S-SPV-089 is a swinepox virus that expresses three
foreign genes. . The gene for *E. coli* β -galactosidase
(*lacZ*) and the gene for feline leukemia virus (FeLV)
gag/protease were inserted into the 738-94.4 ORF (a 773
base pair deletion of the SPV O1L ORF; Deletion of

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nucleotides 1679 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox OIL promoter, and the FeLV gag/protease gene is under the control of the synthetic late/early promoter (LP2EP2).

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S-SPV-089 is derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the HOMOLOGY VECTOR 832-26.A1 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
10 GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 089. This virus was assayed for
15 β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus is
20 pure, stable, and expressing the foreign gene.

S-SPV-089 was assayed for expression of FeLV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE
25 EXPRESSION IN RECOMBINANT SPV. Polyclonal mouse anti-p27 serum, rabbit anti-p27 serum and rabbit anti- β -galactosidase were shown to react specifically with S-SPV-089 plaques and not with S-SPV-003 negative control plaques. All S-SPV-089 observed plaques reacted with the antiserum indicating that the virus was stably
30 expressing the FeLV gag protease and E. coli β -galactosidase proteins.

S-SPV-089 is useful as a vaccine in cats against disease caused by feline leukemia virus. The FeLV
35 gag/protease antigen is key to raising a protective

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immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-089 is also useful as an expression vector for expressing FeLV antigens. Such FeLV antigens are useful to identify antibodies directed against the wild-type FeLV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTOR 832-26.A1. The plasmid 832-26.A1 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lac Z) marker gene and the feline leukemia virus (FeLV) gag/protease gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a swinepox virus 01L gene promoter and the FeLV gag/protease gene is under the control of the late/early promoter (LP2EP2). The LP2EP2 FeLV gag/protease gene cassette was inserted into a EcoRI and BamHI site of homology vector 752-22.1. Homology vector 832-26.A1 was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid

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vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5' GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends.

Fragment 2 is a 3002 base pair BamHI to PvuII fragment derived from plasmid pJF751 (49) containing the *E. coli* lacZ gene. Fragment 3 is an approximately 2160 base pair EcoRI to BamHI restriction fragment of the FeLV gag/protease (gag ORF is approximately 584 amino acids; protease ORF is approximately 136 amino acids) synthesized by polymerase chain reaction (PCR) using cDNA from FeLV/FAIDS strain, Type A (cDNA clone p61E; Dr. Mullens, NIAIDS repository). The primer (5' CGTCGAATTCGATGTCTGGAGCCTCTAGTGGGA-3'; 1/96.32) (SEQ ID NO 38) synthesizes from the 5' end of the FeLV gag/protease gene, introduces an EcoRI site at the 5' end of the gene and an ATG start codon. The primer (5'- CGTCGGATCCGGCTCAAATAGCCGATACTCTTCTT-3'; 1/96.33) (SEQ ID NO 39) synthesizes from the 3' end of the FeLV gag/protease gene. The PCR product was digested with EcoRI and BglII to yield a fragment 2160 base pairs in length corresponding to the FeLV gag/protease gene. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5' GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

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S-SPV-100

S-SPV-100 is a swinepox virus that expresses two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for feline leukemia virus (FeLV) envelope (env) gp70 + p15E were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the FeLV env gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-100 is derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the HOMOLOGY VECTOR 843-9.322 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-100. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-SPV-100 was assayed for expression of FeLV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal mouse anti-p70 serum and rabbit anti- β -galactosidase were shown to react specifically with S-SPV-100 plaques and not with S-SPV-003 negative control plaques. All S-SPV-100 observed plaques reacted with the antiserum indicating

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that the virus was stably expressing the FeLV env and E. coli β -galactosidase proteins.

5 S-SPV-100 is useful as a vaccine in cats against disease caused by feline leukemia virus. The FeLV env antigen is key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-100 is also useful as an expression vector for expressing FeLV
10 antigens. Such FeLV antigens are useful to identify antibodies directed against the wild-type FeLV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the
15 development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

20
HOMOLOGY VECTOR 843-9.322. The plasmid 843-9.322 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and a feline leukemia virus (FeLV)
25 envelope (env) gp70 + p15E gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according
30 to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the FeLV env gene is under
35 the control of the late/early promoter (LP2EP2). It was

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constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an
5 approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 1973
10 base pair EcoRI to BamHI restriction fragment of the FeLV env (gp70 + p15E) (env ORF is approximately 658 amino acids) synthesized by polymerase chain reaction (PCR) using cDNA from FeLV/FAIDS strain, Type A (cDNA clone p61E; Dr. Mullens, NIAIDS repository) The primer
15 (5-'CGTCGAATTCAATGGAAAGTCCAACGCACCCAAAA-3'; 1/96.31) (SEQ ID NO 40) synthesizes from the 5' end of the FeLV env gene, introduces an EcoRI site at the 5' end of the gene and an ATG start codon. The primer (5'-
CGTCGGATCCGGGGACTAAATGGAATCATACA -3'; 1/96.28) (SEQ ID
20 NO 41) synthesizes from the 3' end of the FeLV env gene. The PCR product was digested with EcoRI and BglII to yield a fragment 1973 base pairs in length corresponding to the FeLV env gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII
25 restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers.

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S-SPV-107 and S-SPV-108

S-SPV-107 is a swinepox virus that expresses three foreign genes. The gene E. coli β -galactosidase (lacZ)

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is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for feline leukemia virus (FeLV) gag/protease and envelope (env) gp70 + p15E was
5 inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been
10 deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the FeLV gag/protease and env genes are under the control of the synthetic late/early promoter (LP2EP2). The direction of transcription of the FeLV gag/protease and env gene are
15 the same as direction of transcription of the lacZ gene and the SPV 01L gene.

S-SPV-107 is derived from S-SPV-001 (Kasza Strain). This is accomplished utilizing the HOMOLOGY VECTOR 840-
20 68.A1 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque
25 purification is the recombinant virus designated S-SPV 107. This virus is assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial
30 three rounds of purification, all plaques observed were blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-SPV-108 is a swinepox virus that expresses three
35 foreign genes. The gene E. coli β -galactosidase (lacZ)

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is inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site in the SPV 01L open reading frame). The gene for feline leukemia virus (FeLV) gag/protease and envelope (env) gp70 + p15E was
5 inserted into the unique NotI restriction site (NotI linkers inserted into a unique NdeI site in the SPV 01L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been
10 deleted). The lacZ gene is under the control of the synthetic late promoter (LP1), the FeLV gag/protease and env gene is under the control of the synthetic late/early promoter (LP2EP2). The direction of transcription of the FeLV gag/protease and env gene are
15 opposite the direction of transcription of the lacZ gene and the SPV 01L gene.

S-SPV-108 is derived from S-SPV-001 (Kasza Strain). This is accomplished utilizing the HOMOLOGY VECTOR
20 VECTOR 840-68.A6 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque
25 purification is the recombinant virus designated S-SPV-108. This virus is assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial
30 three rounds of purification, all plaques observed were blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-SPV-107 or S-SPV-108 are useful as a vaccine in cats
35 against disease caused by feline leukemia virus. The

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FeLV gag/protease and env antigens are key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. S-SPV-107 or S-SPV-108 are also useful as an expression vector for expressing FeLV antigens. Such FeLV antigens are useful to identify antibodies directed against the wild-type FeLV. The virus is also useful as a source of antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

HOMOLOGY VECTORS 840-68.A1 AND 840-68.A6. The homology vector are constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and for feline leukemia virus (FeLV) protease (gag) and envelope (env) gp70 + p15E genes flanked by SPV DNA. The direction of transcription of the FeLV gag/protease and env gene is the same as direction of transcription of the LacZ gene and the SPV O1L gene in homology vector 840-68.A1. The direction of transcription of the FeLV gag/protease and env gene is opposite the direction of transcription of the lacZ gene and the SPV O1L gene in 840-68.A6. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the

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β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the FeLV gag/protease and env gene are each under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 48 base pair AccI to NdeI subfragment of the SPV HindIII M fragment. Fragment 4 is an approximately 2160 base pair EcoRI to BamHI restriction fragment of the FeLV gag/protease (gag ORF is approximately 584 amino acids; protease ORF is approximately 136 amino acids) synthesized by polymerase chain reaction (PCR) using cDNA from FeLV/FAIDS strain, Type A (cDNA clone p61E; Dr. Mullens, NIAIDS repository). The primer (5' CGTCGAATTCGATGTCTGGAGCCTCTAGTGGGA-3'; 1/96.32) (SEQ ID NO 38) synthesizes from the 5' end of the FeLV gag/protease gene, introduces an EcoRI site at the 5' end of the gene. One in frame start codon (ATG) is in the LP2EP2 promoter, and a second in frame start codon is in the gene coded by the PCR primer. The primer (5'-CGTCGGATCCGGCTCAAATAGCCGATACTCTTCTT-3'; 1/96.33) (SEQ ID NO 39) synthesizes from the 3' end of the FeLV gag/protease gene. The PCR product was digested with EcoRI and BglII to yield a fragment 2160 base pairs in length corresponding to the FeLV gag/protease gene.

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Fragment 5 is an approximately 1973 base pair EcoRI to BamHI restriction fragment of the FeLV env (gp70 + p15E) (env ORF is approximately 658 amino acids) synthesized by polymerase chain reaction (PCR) using

5 cDNA from FeLV/FAIDS strain, Type A (cDNA clone p61E; Dr. Mullens, NIAIDS repository) The primer (5'-
'CGTCGAATTCAATGGAAAGTCCAACGCACCCAAAA-3'; 1/96.31) (SEQ
ID NO 40) synthesizes from the 5' end of the FeLV env
gene, introduces an EcoRI site at the 5' end of the
10 gene. One in frame start codon (ATG) is in the LP2EP2
promoter, and a second in frame start codon is in the
gene coded by the PCR primer The primer (5'-
CGTCGGATCCGGGGACTAAATGGAATCATACA -3'; 1/96.28) (SEQ ID
NO 41) synthesizes from the 3' end of the FeLV env
15 gene. The PCR product was digested with EcoRI and BglII
to yield a fragment 1973 base pairs in length
corresponding to the FeLV env gene. Fragment 6 is an
approximately 1560 base pair NdeI to HindIII
subfragment of the SPV HindIII fragment M. The AccI
20 sites in fragments 1 and 3 were converted to unique
PstI sites using PstI linkers. The NdeI sites in
fragments 3 and 6 were converted to unique Not I sites
using NotI linkers. An approximately 545 base pair NdeI
to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID
25 NO. 189) of the SPV HindIII M fragment has been deleted
which would span SPV fragments 3 and 6.

S-SPV-128:

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S-SPV-128 is a swinepox virus that expresses four foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for for feline leukemia virus (FeLV) gag/protease and were inserted into the 738-94.4

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ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1669 to 2452, SEQ ID NO: 189). The gene for E. coli β -glucuronidase (uidA) and the gene for the feline leukemia virus (FeLV) envelope (env) gp70 + p15E were inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region (SEQ ID NO 1) of the 6.7 kb SPV HindIII K fragment). The lacZ gene is under the control of the synthetic late promoter (LP1), the uidA gene is under the control of the synthetic early promoter (EP2) and the FeLV gag/protease and envelope genes are each under the control of the synthetic late/early promoter (LP2EP2).

15 S-SPV-128 was derived from S-SPV-089 (Kasza Strain). This was accomplished utilizing the homology vector 860-2 (see Materials and Methods) and virus S-SPV-089 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase and β -glucuronidase (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of red plaque and blue plaque purification was the recombina

20 nt virus designated S-SPV-128. This virus was assayed for β -galactosidase and β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

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S-SPV-128 is useful as a vaccine in cats against FIV infection S-SPV-128 is also useful for expression of the FIV env and gag proteins.

- 5 **HOMOLOGY VECTOR 860-2.** The plasmid 860-2 was used to insert foreign DNA into SPV. It incorporates a gene for E. coli β -glucuronidase (uidA) and the gene for the feline leukemia virus (FeLV) envelope (env) gp70 + p15E flanked by SPV DNA. When this plasmid was used
10 according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β -glucuronidase (uidA) gene is under the control of a synthetic early pox promoter (EP2) and the FeLV env
15 gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA
20 sequences. The plasmid vector was derived from an approximately 3005 base pair HindIII restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 1652 base pair HindIII to EcoRI restriction sub-fragment of the SPV HindIII restriction
25 fragment K. Fragment 2 is an approximately 1973 base pair EcoRI to BamHI restriction fragment of the FeLV env (gp70 + p15E) (approximately 860 amino acids which includes the full length SU and TM coding regions of FIV env) synthesized by polymerase chain reaction (PCR)
30 using cDNA from FeLV/FAIDS strain, Type A (cDNA clone p61E; Dr. Mullens, NIAIDS repository) The primer (5'-CGTCGAATTCAATGGAAAGTCCAACGCACCCAAA-3'; 1/96.31) (SEQ ID NO 40) synthesizes from the 5' end of the FeLV env gene, introduces an EcoRI site at the 5' end of the

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gene and an ATG start codon. The primer (5'-
CGTCGGATCCGGGGACTAAATGGAATCATACA -3'; 1/96.28) (SEQ ID
NO 41) synthesizes from the 3' end of the FeLV env
gene. The PCR product was digested with EcoRI and BglII
5 to yield a fragment 1973 base pairs in length
corresponding to the FeLV env gene.. Fragment 3 is an
approximately 1800 base pair EcoRI to XmaI restriction
fragment containing the *E. coli uidA* gene. Fragment 4
is an approximately 5053 base pair EcoRI to HindIII
10 restriction sub-fragment of the SPV HindIII restriction
fragment K. The EcoRI sites in fragments 1 and 4 of the
SPV homology vector were converted to a unique NotI
site.

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PORCINE CONSTRUCTS AND VACCINES

S-SPV-084

5 S-SPV-084 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF6 were inserted into the SPV 738-94.4 ORF (a 773 base pair deletion of
10 the SPV OIL ORF; Deletion of nucleotides 1669 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox POIL promoter and the PRRS ORF6 gene is under the control of the synthetic late/early promoter (LP2EP2).

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S-SPV-084 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 831-38.22 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
20 GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 084. This virus was assayed for
25 β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus
30 was pure, stable, and expressing the foreign gene.

To confirm the expression of the PRRS ORF6 matrix protein gene product, cells were infected with S-SPV-084 and samples of infected cell lysates were subjected
35 to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING

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PROCEDURE. A polyclonal swine anti-PRRS (NVSL) serum was used to detect expression of PRRS specific proteins. The cell lysate from S-SPV-084 infected cells exhibited a band corresponding to 18 kd, which is the expected size of the PRRS ORF6 matrix protein.

S-SPV-084 is useful as a vaccine in swine against PRRS infection S-SPV-084 is also useful for expression of the PRRS ORF6 matrix protein.

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HOMOLOGY VECTOR 831-38.22. The homology vector 831-38.22 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the porcine reproductive and respiratory syndrome virus (PRRS) ORF6 matrix gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of the swinepox virus O1L promoter and the PRRS ORF6 matrix gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65 (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5' GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce

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an 855 base pair fragment with SphI and BglII ends. Fragment 2 is an approximately 3002 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 3 is an EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the NVSL (Reference strain, IA-2). To synthesize PRRS ORF6 matrix gene, the primer (5' CGGGAATTCGGGGTCGTCCTTAGATGACTTCTGCC-3'; 1/96.17) (SEQ ID NO 42) synthesizes from the 5' end of the PRRS ORF6 gene, introduces an EcoRI site at the 5' end of the gene. The primer (5' GCGGATCCTTGTATGTGGCATATTTGACAAGGTTTAC-3'; 1/96.18) (SEQ ID NO 43) is used for reverse transcription and PCR and synthesizes from the 3' end of the PRRS ORF6 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment 532 base pairs in length corresponding to the PRRS ORF6 gene. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5' - CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5' GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

25

S-SPV-091

S-SPV-091 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF2 were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the PRRS ORF2 gene is under the control of the synthetic late/early promoter (LP2EP2).

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S-SPV-091 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 844-15.110 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
5 GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 091. This virus was assayed for
10 β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus
15 was pure, stable, and expressing the foreign gene.

S-SPV-091 is useful as a vaccine in swine against PRRS infection. S-SPV-091 is also useful for expression of the PRRS ORF2 protein.

20
HOMOLOGY VECTOR 844-15.110. The plasmid 844-15.110 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and a porcine reproductive and
25 respiratory syndrome virus (PRRS) ORF2 gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is
30 used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and
35 the PRRS ORF2 gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30),

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by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the NVSL (Reference strain, IA-2). To synthesize PRRS ORF2, the primer (5' AATGAATTCGAAATGGGGTCCATGCAAAGCCTTTTG-3'; 1/96.15) (SEQ ID NO 44) synthesized from the 5' end of the PRRS ORF2 gene, introduced an EcoRI site at the 5' end of the gene. The primer (5' - CAAGGATCCCACACCGTGTAATTCAGTGTGAGTTCG-3'; 1/96.16) (SEQ ID NO 45) was used for reverse transcription and PCR and synthesized from the 3' end of the PRRS ORF2 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment approximately 788 base pairs in length corresponding to the PRRS ORF2 gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

30 S-SPV-092

S-SPV-092 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF7 nucleocapsid were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction

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site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the PRRS ORF7 nucleocapsid gene is under the control of the synthetic late/early promoter (LP2EP2).

5

S-SPV-092 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 844-19.94 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
10 GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 092. This virus was assayed for
15 β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus
20 was pure, stable, and expressing the foreign gene.

S-SPV-092 was assayed for expression of PRRS-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-
25 PRRS (NVSL) serum and a monoclonal antibody to PRRS ORF7 nucleocapsid protein were each shown to react specifically with S-SPV-092 plaques and not with S-SPV-003 negative control plaques. All S-SPV-092 observed plaques reacted with the antiserum indicating that the
30 virus was stably expressing the PRRS ORF7 nucleocapsid protein.

S-SPV-092 is useful as a vaccine in swine against PRRS infection. S-SPV-092 is also useful for expression of
35 the PRRS ORF7 nucleocapsid protein.

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HOMOLOGY VECTOR 844-19.94. The plasmid 844-19.94 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and a porcine reproductive and respiratory syndrome virus (PRRS) ORF7 gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the PRRS ORF7 gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the NVSL (Reference strain, IA-2). To synthesize PRRS ORF7, the primer (5' GTCGAATTCGCCAAATAACAACGGCAAGCAGCAGAAG 3'; 1/96.19) (SEQ ID NO 46) synthesized from the 5' end of the PRRS ORF7 gene, introduced an EcoRI site at the 5' end of the gene. The primer (5' - CAAGGATCCCAGCCCATCATGCTGAGGGTGATG-3'; 1/96.20) (SEQ ID NO 47) was used for reverse transcription and PCR and synthesized from the 3' end of the PRRS ORF7 gene. The PCR product was digested with EcoRI and BamHI to yield

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a fragment approximately 383 base pairs in length corresponding to the PRRS ORF7 gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

10

S-SPV-093

S-SPV-093 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF3 were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the PRRS ORF3 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-093 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 839-58.9 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 093. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

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S-SPV-093 was assayed for expression of PRRS-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-PRRS (NVSL) serum was shown to react specifically with
5 S-SPV-093 plaques and not with S-SPV-003 negative control plaques. All S-SPV-093 observed plaques reacted with the antiserum indicating that the virus was stably expressing the PRRS ORF3 protein.

10 To confirm the expression of the PRRS ORF3 protein gene product, cells were infected with S-SPV-093 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN
15 BLOTTING PROCEDURE. A polyclonal swine anti-PRRS (NVSL) serum was used to detect expression of PRRS specific proteins. The cell lysate and culture supernatant from S-SPV-093 infected cells exhibited a band corresponding to 45 kd, which is the expected size of the PRRS ORF3
20 protein. ORF3 protein was shown to be secreted from infected cells into the culture media.

S-SPV-093 is useful as a vaccine in swine against PRRS infection. S-SPV-093 is also useful for expression of
25 the PRRS ORF3 protein.

HOMOLOGY VECTOR 839-58.9. The plasmid 839-58.9 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and a porcine reproductive and
30 respiratory syndrome virus (PRRS) ORF3 gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately
35 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus

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containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the PRRS ORF3 gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the NVSL (Reference strain, IA-2). To synthesize PRRS ORF3, the primer 5'-TTCGAATTCGGCTAATAGCTGTACATTCCTCCATATTT-3'; 1/96.7) (SEQ ID NO 48) synthesized from the 5' end of the PRRS ORF3 gene, introduced an EcoRI site at the 5' end of the gene. The primer (5'-GGGGATCCTATCGCCGTACGGCACTGAGGG-3'; 1/96.8) (SEQ ID NO 49) was used for reverse transcription and PCR and synthesized from the 3' end of the PRRS ORF3 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment approximately 768 base pairs in length corresponding to the PRRS ORF3 gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

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S-SPV-094 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF4 were inserted
5 into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the PRRS ORF4 gene is under the control of the synthetic late/early promoter (LP2EP2).

10

S-SPV-094 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 839-58.36 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
15 GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 094. This virus was assayed for
20 β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus
25 was pure, stable, and expressing the foreign gene.

S-SPV-094 was assayed for expression of PRRS-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-
30 PRRS (NVSL) serum was shown to react specifically with S-SPV-094 plaques and not with S-SPV-003 negative control plaques. All S-SPV-094 observed plaques reacted with the antiserum indicating that the virus was stably expressing the PRRS ORF4 protein.

35

To confirm the expression of the PRRS ORF4 protein gene product, cells were infected with S-SPV-094 and samples

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of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRRS (NVSL) serum was used to
5 detect expression of PRRS specific proteins. The cell lysate from S-SPV-094 infected cells exhibited a band corresponding to 31 kd, which is the expected size of the PRRS ORF4 protein (202 amino acids).

10 S-SPV-094 is useful as a vaccine in swine against PRRS infection. S-SPV-094 is also useful for expression of the PRRS ORF4 protein.

HOMOLOGY VECTOR 839-58.36. The plasmid 839-58.36 was
15 constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and a porcine reproductive and respiratory syndrome virus (PRRS) ORF4 gene flanked by SPV DNA. Upstream of the foreign gene is an
20 approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus
25 containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the PRRS ORF4 gene is under the control of the late/early promoter (LP2EP2). It was constructed
30 utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64
35 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an

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EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the NVSL (Reference strain, IA-2). To
5 synthesize PRRS ORF4, the primer (5'-
CCGAATTCGGCTGCGTCCCTTCTTTTCCTCATGG-3'; 1/96.11) (SEQ ID
NO 50) synthesized from the 5' end of the PRRS ORF4
gene, introduced an EcoRI site at the 5' end of the
gene. The primer (5'
10 CTGGATCCTTCAAATTGCCAACAGAATGGCAAAAAGAC-3'; 1/96.12)
(SEQ ID NO 51) was used for reverse transcription and
PCR and synthesized from the 3' end of the PRRS ORF4
gene. The PCR product was digested with EcoRI and BamHI
to yield a fragment approximately 542 base pairs in
15 length corresponding to the PRRS ORF4 gene. Fragment 3
is an approximately 3010 base pair BamHI to PvuII
restriction fragment of plasmid pJF751 (11). Fragment
4 is an approximately 2149 base pair AccI to HindIII
subfragment of the SPV HindIII fragment M. The AccI
20 sites in fragments 1 and 4 were converted to unique
NotI sites using NotI linkers.

S-SPV-095

25 S-SPV-095 is a swinepox virus that expresses at least
two foreign genes. The gene for E. coli β -galactosidase
(lacZ) and the gene for porcine reproductive and
respiratory syndrome virus (PRRS) ORF5 were inserted
into the SPV 617-48.1 ORF (a unique NotI restriction
30 site has replaced a unique AccI restriction site). The
lacZ gene is under the control of the synthetic late
promoter (LP1), and the PRRS ORF5 gene is under the
control of the synthetic late/early promoter (LP2EP2).
35 S-SPV-095 was derived from S-SPV-001 (Kasza Strain).
This was accomplished utilizing the homology vector
839-58.43 (see Materials and Methods) and virus S-SPV-

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001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
GENERATING RECOMBINANT SPV. The transfection stock was
screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING
 β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final
5 result of red plaque purification was the recombinant
virus designated S-SPV 095. This virus was assayed for
 β -galactosidase expression, purity, and insert
stability by multiple passages monitored by the blue
plaque assay as described in Materials and Methods.
10 After the initial three rounds of purification, all
plaques observed were blue indicating that the virus
was pure, stable, and expressing the foreign gene.

To confirm the expression of the PRRS ORF5 protein gene
15 product, cells were infected with S-SPV-095 and samples
of infected cell lysates were subjected to SDS
polyacrylamide gel electrophoresis. The gel was blotted
and analyzed using the WESTERN BLOTTING PROCEDURE. A
polyclonal swine anti-PRRS (NVSL) serum was used to
20 detect expression of PRRS specific proteins. The cell
lysate from S-SPV-095 infected cells exhibited a band
corresponding to 26 kd, which is the expected size of
the PRRS ORF5 protein.

25 S-SPV-095 is useful as a vaccine in swine against PRRS
infection. S-SPV-095 is also useful for expression of
the PRRS ORF5 protein.

HOMOLOGY VECTOR 839-58.43. The plasmid 839-58.43 was
30 constructed for the purpose of inserting foreign DNA
into SPV. It incorporates an E. coli β -galactosidase
(lacZ) marker gene and a porcine reproductive and
respiratory syndrome virus (PRRS) ORF5 gene flanked by
SPV DNA. Upstream of the foreign gene is an
35 approximately 1484 base pair fragment of SPV DNA.
Downstream of the foreign genes is an approximately
2149 base pair fragment of SPV DNA. When the plasmid is

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used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the PRRS ORF5 gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the NVSL (Reference strain, IA-2). To synthesize PRRS ORF5, the primer (5'-TTGAATTCGTTGGAGAAATGCTTGACCGCGGGC-3'; 1/96-13) (SEQ ID NO 52) synthesized from the 5' end of the PRRS ORF5 gene, introduced an EcoRI site at the 5' end of the gene. The primer (5'-GAAGGATCCTAAGGACGACCCCATTGTTCCGCTG-3'; 1/96-14) (SEQ ID NO 53) was used for reverse transcription and PCR and synthesized from the 3' end of the PRRS ORF5 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment approximately 606 base pairs in length corresponding to the PRRS ORF5 gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

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S-SPV-076

S-SPV-076 is a swinepox virus that expresses at least
5 two foreign genes. The gene for E. coli β -galactosidase
(lacZ) and the gene for pseudorabies virus (PRV)
glycoprotein D (gD) and glycoprotein I (gI) were
inserted into the SPV 617-48.1 ORF (a unique NotI
10 restriction site has replaced a unique AccI restriction
site). The lacZ gene is under the control of the
synthetic late promoter (LP1), and the PRV gD and gI
gene is under the control of the synthetic late/early
promoter (LP2EP2).

15 S-SPV-076 was derived from S-SPV-001 (Kasza Strain).
This was accomplished utilizing the homology vector
829-55.16 (see Materials and Methods) and virus S-SPV-
001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
GENERATING RECOMBINANT SPV. The transfection stock was
20 screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING
 β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final
result of red plaque purification was the recombinant
virus designated S-SPV-076. This virus was assayed for
 β -galactosidase expression, purity, and insert
25 stability by multiple passages monitored by the blue
plaque assay as described in Materials and Methods.
After the initial three rounds of purification, all
plaques observed were blue indicating that the virus
was pure, stable, and expressing the foreign gene.

30 S-SPV-076 was assayed for expression of PRV-specific
antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE
EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-
PRV serum was shown to react specifically with S-SPV-
35 076 plaques and not with S-SPV-003 negative control
plaques. All S-SPV-076 observed plaques reacted with

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the antiserum indicating that the virus was stably expressing the PRV gD protein.

5 S-SPV-076 is useful as a vaccine in swine against PRV infection. S-SPV-076 is also useful for expression of the PRV gD and gI proteins.

HOMOLOGY VECTOR 829-55.16. The plasmid 829-55.16 was constructed for the purpose of inserting foreign DNA
10 into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and a pseudorabies virus (PRV) glycoprotein D (gD) and glycoprotein I (gI) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 1484 base pair fragment of SPV DNA.
15 Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will
20 result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the PRV gD and gI genes are under the control of the late/early promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA
25 techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is
30 an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 500 base pair EcoRI to SalI restriction fragment derived from plasmid 538-46.16 (See WO95/03070). Fragment 3 is
35 an approximately 1900 base pair SalI to BamHI restriction subfragment of PRV BamHI#7 genomic DNA fragment. Fragment 4 is an approximately 3010 base

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pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 5 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 5 were converted to unique NotI sites using NotI linkers.

S-SPV-079

10 S-SPV-079 is a swinepox virus that expresses two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for pseudorabies virus (PRV) glycoprotein B (gB) were inserted into the unique HindIII restriction site (HindIII linkers inserted into
15 a unique NdeI site in the SPV O1L open reading frame; An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted). The lacZ gene is under the control of the synthetic late promoter (LP1),
20 the PRV gB gene is under the control of the synthetic late/early promoter (LP2EP2). S-SPV-079 contains a PRV gB gene which codes for a protein of 913 amino acids, including 69 amino acids at the carboxy terminus which are missing in the PRV gB gene of S-SPV-015.

25 S-SPV-079 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 825-84.3 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
30 GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV 079. This virus was assayed for
35 β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods.

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After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

5 S-SPV-079 was assayed for expression of PRV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-PRV serum was shown to react specifically with S-SPV-079 plaques and not with S-SPV-003 negative control
10 plaques. All S-SPV-079 observed plaques reacted with the antiserum indicating that the virus was stably expressing the PRV gB protein.

To confirm the expression of the PRV gB gene product,
15 cells were infected with S-SPV-079 and samples of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRV serum was used to detect
20 expression of PRV specific proteins. The cell lysate from S-SPV-079 infected cells exhibited bands corresponding to 120 kd gB precursor and the 67 kd and 58 kd processed forms, which are the expected size of the PRV gB protein. PRV gB exists as a disulfide linked
25 complex of these three forms.

S-SPV-079 is useful as a vaccine in swine against PRV infection. S-SPV-079 is also useful for expression of the PRV gB protein.

30

HOMOLOGY VECTOR 825-84.3. The plasmid 825-84.3 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the pseudorabies virus (PRV)
35 glycoprotein B (gB) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1532 base pair fragment of SPV DNA. Downstream of the foreign genes is

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an approximately 1560 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), the PRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1532 base pair BglII to NdeI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 2600 base pair EcoRI to SalI fragment derived from plasmid 727-54.60. Fragment 2 contains approximately 43 base pairs of synthetic DNA coding for PRV gB amino acids 1 to 16 and an approximately 2600 base pair SmaI to SalI fragment of PRV KpnI C genomic DNA. Fragment 3 is an approximately 210 base pair SalI to BamHI fragment generated by PCR which contains the coding sequence of the PRV gB gene. Fragment 3 contains the carboxy terminal 69 amino acids of PRV gB which are missing from S-SPV-015. Fragment 3 is an SalI to BamHI restriction fragment synthesized by polymerase chain reaction (PCR) using template DNA from PRV KpnI C genomic DNA. The primer (5' ATGAAGGCCCTGTACCCCGTCACGA-3'; 11/95.3) (SEQ ID NO 54) synthesized across the SalI of the PRV gB gene and reproduced a SalI site internal to the gB gene. The primer (5'-CGGGATCCGGCTACAGGGCGTCGGGGTCCTC3'-3'; 11/95.4) (SEQ ID NO 55) was used for PCR and synthesized from the 3' end of the PRV gB gene and introduced a BamHI site at the 3' end of the PRV gB gene. The PCR product was digested

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with SalI and BamHI to yield a fragment approximately 210 base pairs in length corresponding to the carboxy terminus of the PRV gB gene. Fragment 4 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 5 is an approximately 1560 base pair NdeI to HindIII subfragment of the SPV HindIII fragment M. The NdeI sites in fragments 1 and 5 were converted to unique HindIII sites using HindIII linkers. An approximately 545 base pair NdeI to NdeI subfragment (Nucleotides 1560 to 2104; SEQ ID NO. 189) of the SPV HindIII M fragment has been deleted which would span SPV fragments 1 and 5.

15

S-SPV-090:

S-SPV-090 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ) and the gene for pseudorabies virus (PRV) glycoprotein I (gI) were inserted into the 738-94.4 ORF (a 773 base pair deletion of the SPV O1L ORF; Deletion of nucleotides 1679 to 2452, SEQ ID NO: 189). The lacZ gene is under the control of the swinepox O1L promoter, and the PRV gI gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-090 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 837-58.14 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-090. This virus was assayed for β -galactosidase expression, purity, and insert

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stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-090 is useful as a vaccine in swine against PRV infection. S-SPV-090 is also useful for expression of the PRV gI protein.

10

HOMOLOGY VECTOR 837-58.14. The plasmid 837-58.14 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lac Z) marker gene and an pseudorabies virus (PRV) glycoprotein I (gI) gene flanked by SPV DNA. Upstream of the foreign gene is an approximately 855 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1113 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the β galactosidase (lacZ) marker gene is under the control of a swinepox virus 01L gene promoter and the PRV gI gene is under the control of the late/early promoter (LP2EP2). The LP2EP2 PRV gI gene cassette was inserted into a NotI site of homology vector 752-22.1. Homology vector 840-72.A1 was constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2519 base pair HindIII to SphI restriction fragment of pSP65. (Promega). Fragment 1 is an approximately 855 base pair sub-fragment of the SPV HindIII restriction fragment M (23) synthesized by polymerase chain reaction using DNA primers 5' GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' and 5'-

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CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' to produce an 855 base pair fragment with SphI and BglII ends. Fragment 2 is a 3002 base pair BamHI to PvuII fragment derived from plasmid pJF751 (49) containing the *E. coli* lacZ gene. Fragment 3 is an approximately 1150 base pair BamHI fragment coding for the PRV gI gene derived by polymerase chain reaction (PCR) (Sambrook, et al., 1989) using the PRV BamHI#7 DNA fragment (pSY 138.-09.W) as template for the PCR reaction. To synthesize PRV gI, the primer (5'-CCGGATCCGGCGCGCGACGTGACCCGGCTC-3'; 11/95.1) (SEQ ID NO 56) synthesized from the 5' end of the PRV gI gene and introduced a BamHI site at the 5' end of the gene. The primer (5'-CCGGATCCGGCGGACGGAGATAAAACGCCACCCAC -3'; 11/95.2) (SEQ ID NO 57) synthesized from the 3' end of the PRV gI gene and introduced a BamHI site at the 3' end of the gene. The PCR product was digested with BamHI to yield a fragment approximately 1150 base pairs in length corresponding to the PRV gI gene. Fragment 4 is an approximately 1113 base pair subfragment of the SPV HindIII fragment M synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' and 5'-GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' to produce an 1113 base pair fragment with SalI and HindIII ends.

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Example 44: Homology Vectors Useful for Inserting Foreign DNA into the SPV HindIII K Genomic Region of a Recombinant Swinepox Virus

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Plasmid 854-90.1 was constructed for insertion of foreign DNA into a recombinant swinepox virus. Plasmid 854-90.1 was constructed by changing the unique Eco RI site within the SPV Hind III K genomic fragment (Nucleotides: SEQ ID NO: 1) to a unique Not I restriction site through use of a DNA linker. The homology vector 854-90.1 contains an 1652 base pair region of SPV DNA upstream of the Not I insertion site and 5058 base pair region of SPV DNA downstream of the Not I insertion site. A homology vector containing foreign DNA inserted into plasmid 854-90.1 is useful when combined with swinepox virus DNA by HOMOLOGOUS RECOMBINATION for the construction of recombinant swinepox viruses.

20

Plasmid 855-37.5 was constructed for insertion of foreign DNA into a recombinant swinepox virus. Plasmid 855-37.5 was constructed by inserting an approximately 1875 base pair Dra I restriction fragment within swinepox virus HindIII K genome fragment from plasmid 854-90.1 containing the unique Not I insertion site, into plasmid PNEB193. The homology vector 855-37.5 contains an approximately 881 base pair region of SPV DNA upstream of the Not I insertion site and an approximately 994 base pair region of SPV DNA downstream of the Not I insertion site. The total size of homology vector 855-37.5 is approximately 3.9 kb making it ideal for the insertion of two or more foreign genes into the homology vector and by homologous recombination into a recombinant swinepox virus.

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Plasmid 847-42.2C was constructed for insertion of foreign DNA into a recombinant swinepox virus. Plasmid 847-42.2C was constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic pox promoter, EP2. Additional foreign DNA is inserted upstream of the uidA gene into unique restriction sites NotI, SfiI and XhoI.

Plasmid 847-42.7B was constructed for insertion of foreign DNA into a recombinant swinepox virus. Plasmid 847-42.2C was constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter, EP2. Additional foreign DNA is inserted downstream of the uidA gene into unique restriction sites NotI, SfiI and XhoI.

S-SPV-120:

S-SPV-120 is a swinepox virus that expresses two foreign genes. The gene for E. coli β -galactosidase (lacZ) was inserted a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment. The gene for E. coli β -glucuronidase (uidA) was inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region (SEQ ID NO: 1) of the 6.7 kb SPV HindIII K fragment). The lacZ gene is under the control of the synthetic late promoter (LP1), the uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-120 was derived from S-SPV-003 (Kasza Strain). This was accomplished utilizing the homology vector 847-42.2C (see above) and virus S-SPV-003 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING

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RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase or β -glucuronidase (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of red plaque purification was the recombinant virus designated S-SPV 120. This virus was assayed for β -galactosidase and β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

S-SPV-120 is useful for inserting additional foreign DNA sequences into recombinant swinepox virus using white plaque selection in the presence of BLUOGAL or X-GLUC to selection for foreign DNA insertion into the unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment and the unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment.

Some examples of recombinant swinepox viruses expressing foreign DNA in the unique AccI restriction site in the O1L ORF of the HindIII M fragment and the unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment are: Recombinant SPV expressing swine influenza virus hemagglutinin, neuraminidase, and nucleoprotein; Recombinant SPV expressing porcine reproductive and respiratory disease virus ORF 5 and ORF6; Recombinant SPV expressing porcine reproductive and respiratory disease virus ORF2, ORF3, ORF4, ORF 5 and ORF6; Recombinant SPV expressing feline immunodeficiency virus gag/protease and envelope; Recombinant SPV

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expressing feline leukemia virus gag/protease and envelope; Recombinant SPV expressing feline immunodeficiency virus gag/protease and envelope and feline leukemia virus gag/protease and envelope;
5 Recombinant SPV expressing infectious bovine rhinotracheitis glycoprotein B, glycoprotein D, and glycoprotein I.

Additional examples of foreign DNA insertion sites in
10 recombinant swinepox virus are exemplified by, but not limited to, one or more of the following: the unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment; the unique NdeI restriction site in the O1L ORF of the SPV HindIII M fragment (See Example S-SPV-
15 052); the unique BglII restriction site within the 2.0 kb BglII to HindIII subfragment of the SPV HindIII M fragment (See Example S-SPV-047); the unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment (See Example S-
20 SPV-059); the unique XhoI restriction site within the SPV HindIII J fragment (See Example S-SPV-064); the unique BglII restriction site within the SPV HindIII N fragment (See Example S-SPV-062); the unique EcoRV restriction site within the SPV HindIII N fragment (See
25 Example S-SPV-060); the unique SnaBI restriction site within the SPV HindIII N fragment (See Example S-SPV-061).

**Example 45: Recombinant swinepox virus expressing swine
30 influenza virus genes in the SPV HindIII M and SPV HindIII K insertion sites**

A recombinant swinepox virus expresses four foreign genes. The gene for swine influenza virus (SIV) hemagglutinin (HA) (H1N1) and the gene for E. coli β -galactosidase (lacZ) were inserted into the SPV 617-
35 48.1 ORF (a unique NotI restriction site has replaced

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a unique AccI restriction site). The gene for swine influenza virus (SIV) neuraminidase (NA) and the gene for E. coli β -glucuronidase (uidA) were inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region (SEQ ID NO: 1) of the 6.7 kb SPV HindIII K fragment). The SIV HA (H1N1) gene is under the control of the synthetic late/early promoter (LP2EP2), the SIV NA gene is under the control of the synthetic late/early promoter (LP2EP2), the lacZ gene is under the control of the synthetic late promoter (LP1), and the uidA gene is under the control of the synthetic early promoter (EP2).

15 The recombinant swinepox virus expressing swine influenza virus genes in the SPV HindIII M and SPV HindIII K insertion sites is derived from S-SPV-065 (Kasza Strain). This is accomplished utilizing the homology vector (with the SIV NA and E. coli uidA genes inserted into a unique NotI site in plasmid 855-37.5 (see above)) and virus S-SPV-065 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase or β -glucuronidase (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of red plaque and blue plaque purification is the recombinant swinepox virus. This virus was assayed for β -galactosidase and β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

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Recombinant swinepox virus expressing swine influenza virus genes in the SPV HindIII M and SPV HindIII K insertion sites is useful as a vaccine in swine against SIV infection and is also useful for expression of the SIV HA and NA proteins.

S-SPV-121:

S-SPV-121 is a swinepox virus that expresses at least one foreign gene. The gene for swine influenza virus (SIV) hemagglutinin (HA) (H1N1) was inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region (SEQ ID NO: 1) of the 6.7 kb SPV HindIII K fragment). The SIV HA gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-121 is derived from S-SPV-059 (Kasza Strain). This is accomplished utilizing the homology vector with the SIV HA gene into a unique NotI site in plasmid 855-37.5 (see above) and virus S-SPV-059 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING ENZYMATIC MARKER GENES (X-GLUC ASSAY). The final result of white plaque purification is the recombinant virus designated S-SPV-121. This virus is assayed for the absence of β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are white indicating that the virus is pure, stable, and expressing the foreign genes.

S-SPV-121 is useful as a vaccine in swine against SIV infection S-SPV-121 is also useful for expression of the SIV HA protein.

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S-SPV-122:

S-SPV-122 is a swinepox virus that expresses two
 5 foreign genes. The gene for swine influenza virus (SIV)
 hemagglutinin (HA) (H1N1) and neuraminidase (NA) were
 inserted into a unique NotI site (NotI linkers inserted
 into a unique EcoRI restriction site within an
 approximately 3.2 kb region (SEQ ID NO: 1) of the 6.7
 10 kb SPV HindIII K fragment). The SIV HA gene is under
 the control of the synthetic late/early promoter
 (LP2EP2) and the SIV NA gene is under the control of
 the synthetic early late promoter (EP2LP2).

15 S-SPV-122 is derived from S-SPV-059 (Kasza Strain).
 This is accomplished utilizing the homology vector with
 the SIV HA and NA genes inserted into a unique NotI
 site in plasmid 855-37.5 (see above) and virus S-SPV-
 059 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
 20 GENERATING RECOMBINANT SPV. The transfection stock was
 screened by the SCREEN FOR RECOMBINANT HERPESVIRUS
 EXPRESSING ENZYMATIC MARKER GENES. The final result of
 white plaque purification is the recombinant virus
 designated S-SPV-122. This virus is assayed for the
 25 absence of β -glucuronidase expression, purity, and
 insert stability by multiple passages monitored by the
 blue plaque assay as described in Materials and
 Methods. After the initial three rounds of
 purification, all plaques observed are white indicating
 30 that the virus is pure, stable, and expressing the
 foreign genes.

S-SPV-122 is useful as a vaccine in swine against SIV
 infection S-SPV-122 is also useful for expression of
 35 the SIV HA and NA proteins.

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Example 46: Recombinant swinepox virus expressing porcine reproductive and respiratory syndrome virus genes in the SPV HindIII M and SPV HindIII K insertion sites

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A recombinant swinepox virus expresses four foreign genes. The gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF5 and the gene for E. coli β -galactosidase (lacZ) were inserted into the
10 SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF6 and the gene for E. coli β -glucuronidase (uidA) were inserted into a unique NotI site (NotI
15 linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region (SEQ ID NO: 1) of the 6.7 kb SPV HindIII K fragment). The PRRS ORF5 gene is under the control of the synthetic late/early promoter (LP2EP2), the PRRS ORF6 gene is under the
20 control of the synthetic late/early promoter (LP2EP2), the lacZ gene is under the control of the synthetic late promoter (LP1), and the uidA gene is under the control of the synthetic early promoter (EP1),

25 The recombinant swinepox virus expressing porcine reproductive and respiratory syndrome virus virus genes in the SPV HindIII M and SPV HindIII K insertion sites is derived from S-SPV-095 (Kasza Strain). This is accomplished utilizing the homology vector (with the
30 PRRS ORF6 and E. coli uidA genes inserted into a unique NotI site in plasmid 855-37.5 (see above)) and virus S-SPV-095 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING
35 β -galactosidase or β -glucuronidase (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of

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red plaque and blue plaque purification is the recombinant swinepox virus. This virus was assayed for β -galactosidase and β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

10

A recombinant swinepox virus expresses four foreign genes. The gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF6 and the gene for E. coli β -galactosidase (lacZ) are inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site.) The gene for porcine reproductive and respiratory system virus (PRRS) ORF5 and the gene for E. coli β -glucuronidase (uidA) are inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region (SEQ ID NO 1) of the 6.7 kb SPV HindIII k fragment). The PRRS ORF6 gene is under the control of the synthetic late/early promoter (LP2EP2), the PRRS ORF5 gene is under the control of the synthetic late/early promoter (LP2EP2), the lacZ gene is under the control of the synthetic late promoter (LP1), and the uidA gene is under the control of the synthetic early promoter (EP2).

30

The recombinant swinepox virus expressing porcine reproductive and respiratory syndrome virus virus genes in the SPV HindIII M and SPV HindIII K insertion sites is derived from S-SPV-084 (Kasza Strain). This is accomplished utilizing the homology vector (with the PRRS ORF5 and E. coli uidA genes inserted into a unique NotI site in plasmid 855-37.5 (see above) and virus S-SPV-084 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR

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GENERATING RECOMBINANT SPV. The transfection stock in screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-galactosidase or B-glucuronidase (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of red plaque and blue plaque purification is the recombinant swinepox virus. This virus is assayed for B-galactosidase and B-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

The recombinant swinepox virus expressing porcine reproductive and respiratory syndrome virus genes in the SPV HindIII M and SPV HindIII K insertion sites is useful as a vaccine in swine against PRRS infection and is also useful for expression of the PRRS ORF5 and ORF6 protein.

Example 47: Recombinant swinepox virus expressing bovine viral diarrhea virus type 1 and type 2 genes in the SPV HindIII M and SPV HindIII K insertion sites

S-SPV-132

S-SPV-132 is a recombinant swinepox virus which expresses four foreign genes. The gene for bovine viral diarrhea virus type 1 (BVDV-1) glycoprotein 53 (gp53) and the gene for E. coli B-galactosidase (lacZ) are inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site.) The gene for bovine viral diarrhea virus type 2 (BVDV-2) glycoprotein 53 (gp53) and the gene for E. coli B-glucuronidase (uidA) are inserted into a unique

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NotI site (NotI linkers are inserted into a unique EcoRI restriction site within an approximately 3.2 kb region (SEQ ID NO 1) OF THE 6.7 KB SPV HindIII K fragment). The BVDV-1 gp53 gene and the BVDV-2 gp53 gene are under the control of the synthetic late/early promoter (LP2EP2), the lacZ gene is under the control of the synthetic late promoter (LP1), and the uidA gene is under the control of the synthetic early promoter (EP2).

10

S-SPV-132 is derived from S-SPV-051 (Kasza Strain). This is accomplished utilizing the homology vector (with the BVDV-2 gp53 and E. coli uidA genes inserted into a unique NotI site in plasmid 855-37.5 (see above)) and virus S-SPV-051 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-galactosidase or B-glucuronidase (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of red plaque and blue plaque purification is the recombinant swinepox virus. This virus is assayed for B-galactosidase and B-glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

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S-SPV-132 is useful as a vaccine in swine against BVDV infection and is also useful for expression of the BVDV-1 gp53 and BVDV-2 gp53.

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S-SPV-134:

S-SPV-134 is a swinepox virus that expresses four foreign genes. The gene for porcine reproductive and

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respiratory syndrome virus (PRRS) ORF6 and the gene for E. coli β -galactosidase (lacZ) were inserted into a unique Not I restriction site (NotI linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF5 and the gene for E. coli β -glucuronidase (uidA) were inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The porcine reproductive and respiratory syndrome virus (PRRS) ORF6 gene is under the control of the synthetic late/early promoter (LP2EP2), the porcine reproductive and respiratory syndrome virus (PRRS) ORF5 gene is under the control of the synthetic late/early promoter (LP2EP2). The lacZ gene is under the control of the synthetic late promoter (LP1), the uidA gene is under the control of the synthetic early promoter (EP2).

20

S-SPV-134 was derived from S-SPV-084 (Kasza Strain). This was accomplished utilizing the homology vector 855-52.31 and virus S-SPV-084 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -glucuronidase (SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of blue plaque purification was the recombinant virus designated S-SPV 134. This virus was assayed for β -galactosidase and β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

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To confirm the expression of the porcine reproductive and respiratory syndrome virus (PRRS) ORF5 and ORF6 gene products, cells were infected with S-SPV-134 and samples of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRRS (NVSL) serum was used to detect expression of PRRS specific proteins. The cell lysate from SPV-134 infected cells exhibited bands corresponding to 26 kd and 18 kd, which is the expected size of the SIV ORF5 and ORF6 protein. A 40 kd band, representing a heterodimer formed between ORF5 and ORF6 protein, was also seen under nonreducing conditions suggesting the formation of a disulfide-linked complex between ORF5 and ORF6 proteins. The assay described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-134 is a recombinant swinepox virus expressing the PRRS ORF5 and ORF6 proteins and is useful as a vaccine in swine against PRRS infection. S-SPV-134 is also useful for expression of the PRRS ORF5 and ORF6 proteins.

HOMOLOGY VECTOR 855-52.31. The homology vector 855-52.31 was used to insert foreign DNA into SPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene and the porcine reproductive and respiratory syndrome virus (PRRS) ORF5 gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β -glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2) and the porcine reproductive and respiratory syndrome virus (PRRS) ORF5 gene is

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under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 855-52.31 was constructed using plasmid 847-42.7C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment.

10 The uidA gene is under the control of the synthetic early promoter (EP2). The PRRS ORF5 gene was then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 855-52.31. The transcriptional and translational orientation of the

15 PRRS ORF5 gene is the same as the uidA gene.

The PRRS ORF5 gene is an approximately 603 base pair EcoRI to BamHI fragment synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the PRRS NVSL reference strain.

20 T h e u p s t r e a m p r i m e r (5'-GAAGGATCCTAAGGACGACCCCATTTGTTCCGCTG-3') synthesizes from the 5' end of the PRRS ORF5 gene and introduces an EcoRI site at the 5' end of the gene. The downstream

25 p r i m e r w a s (5'-GCGGATCCTTGTTATGTGGCATATTTGACAAGGTTTAC-3') synthesizes from the 3' end of the PRRS ORF5 gene, introduces an BamHI site at the 3' end of the gene, and was used for reverse transcription and polymerase chain

30 reaction. The PCR product was digested with EcoRI and BamHI to yield a fragment 603 base pairs in length corresponding to the PRRS ORF5 gene.

35 S-SPV-136 :

S-SPV-136 is a swinepox virus that expresses four foreign genes. The gene for porcine reproductive and

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respiratory syndrome virus (PRRS) ORF6 and the gene for E. coli β -galactosidase (lacZ) were inserted into a unique Not I restriction site (NotI linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF5 and the gene for E. coli β -glucuronidase (uidA) were inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The porcine reproductive and respiratory syndrome virus (PRRS) ORF6 gene is under the control of the synthetic late/early promoter (LP2EP2), the porcine reproductive and respiratory syndrome virus (PRRS) ORF5 gene is under the control of the synthetic late/early promoter (LP2EP2). The lacZ gene is under the control of the synthetic late promoter (LP1), the uidA gene is under the control of the synthetic early promoter (EP2).

20

S-SPV-136 was derived from S-SPV-084 (Kasza Strain). This was accomplished utilizing the homology vector 855-52.43 and virus S-SPV-084 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -glucuronidase (SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of blue plaque purification was the recombinant virus designated S-SPV 136. This virus was assayed for β -galactosidase and β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

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To confirm the expression of the porcine reproductive and respiratory syndrome virus (PRRS) ORF5 and ORF6 gene products, cells were infected with S-SPV-136 and samples of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRRS (NVSL) serum was used to detect expression of PRRS specific proteins. The cell lysate from SPV-136 infected cells exhibited bands corresponding to 26 kd and 18 kd, which is the expected size of the SIV ORF5 and ORF6 protein. A 40 kd band, representing a heterodimer formed between ORF5 and ORF6 protein, was also seen under nonreducing conditions suggesting the formation of a disulfide-linked complex between ORF5 and ORF6 proteins. The assay described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-136 is a recombinant swinepox virus expressing the PRRS ORF5 and ORF6 proteins and is useful as a vaccine in swine against PRRS infection. S-SPV-136 is also useful for expression of the PRRS ORF5 and ORF6 proteins.

25

HOMOLOGY VECTOR 855-52.43. The homology vector 855-52.43 was used to insert foreign DNA into SPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene and the porcine reproductive and respiratory syndrome virus (PRRS) ORF5 gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β -glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2) and the porcine reproductive

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and respiratory syndrome virus (PRRS) ORF5 gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 855-52.43 was constructed using plasmid 847-42.7C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter (EP2). The PRRS ORF5 gene was then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 855-52.31. The transcriptional and translational orientation of the PRRS ORF5 gene is opposite of the uidA gene. The PRRS ORF5 gene is an approximately 603 base pair EcoRI to BamHI fragment synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the PRRS NVSL reference strain. The upstream primer (5'-GAAGGATCCTAAGGACGACCCCATTTGTTCCGCTG-3') synthesizes from the 5' end of the PRRS ORF5 gene and introduces an EcoRI site at the 5' end of the gene. The downstream primer (5'-GCGGATCCTTGTTATGTGGCATATTTGACAAGGTTTAC-3') synthesizes from the 3' end of the PRRS ORF5 gene, introduces an BamHI site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI and BamHI to yield a fragment 603 base pairs in length corresponding to the PRRS ORF5 gene.

S-SPV-157:

S-SPV-157 is a swinepox virus that expresses three foreign genes. The gene for swine influenza virus (SIV) neuraminidase (NA) (H1N1) and the gene for E. coli

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5 β -galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for swine influenza virus (SIV) hemagglutinin (HA) (H1N1) was inserted into a unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The NA gene is under the control of the synthetic late/early promoter (LP2EP2), The HA gene is under the control of the synthetic late/early promoter (LP2EP2), and the lacZ gene is under the control of the synthetic late promoter (LP1).

15 S-SPV-157 was derived from S-SPV-121 (Kasza Strain). This was accomplished utilizing the homology vector 807-84.35 and virus S-SPV-121 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of red plaque purification was the recombinant virus designated S-SPV-157. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

35 To confirm the expression of the SIV HA and NA gene products, cells were infected with S-SPV-157 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal goat

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anti-HA or a polyclonal goat anti-NA serum was used to detect expression of SIV specific proteins. The cell lysate from SPV-157 infected cells exhibited bands corresponding to 64 kd and 52 kd, which is the expected size of the SIV HA and NA protein.

S-SPV-157 was assayed for expression of SIV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-SIV or polyclonal goat anti-HA serum was shown to react specifically with S-SPV-157 plaques and not with S-SPV-001 negative control plaques. All S-SPV-157 observed plaques reacted with both serums indicating that the virus was stably expressing the SIV foreign genes. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-157 is a recombinant swinepox virus expressing the SIV HA and NA proteins and is useful as a vaccine in swine against SIV infection. S-SPV-157 is also useful for expression of the SIV HA and NA proteins.

HOMOLOGY VECTOR 807-84.35. The homology vector 807-84.35 was used to insert foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the swine influenza virus (SIV) neuraminidase (NA) gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the SIV NA gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard

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recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 2 is an approximately 1414 base pair EcoRI to BglII fragment of the SIV NA gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the SIV H1N1 strain (NVSL). The upstream primer (5'-AATGAATTCAAATCAAAAAATAATAACCATTGGGTCAAT-3') synthesizes from the 5' end of the SIV NA gene and introduces an EcoRI site at the 5' end of the gene. The downstream primer was (5'-GGAAGATCTACTTGTCAATGGTGAATGGCAGATCAG-3') synthesizes from the 3' end of the SIV NA gene, introduces an BglII site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI and BglII to yield a fragment 1414 base pairs in length corresponding to the SIV NA gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology vector was converted to a unique NotI site using synthetic linkers.

S-SPV-158:

S-SPV-158 is a swinepox virus that expresses four foreign genes. The gene for swine influenza virus (SIV) nucleoprotein (NP) (H1N1) and the gene for E. coli β -galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a

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unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The genes for swine influenza virus (SIV) hemagglutinin (HA) (H1N1) and neuraminidase (NA) (H1N1) were inserted into a unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The NA gene is under the control of the synthetic late/early promoter (LP2EP2), the HA gene is under the control of the synthetic late/early promoter (LP2EP2), the NP gene is under the control of the synthetic late/early promoter (LP2EP2), and the lacZ gene is under the control of the synthetic late promoter (LP1).

S-SPV-158 was derived from S-SPV-122 (Kasza Strain). This was accomplished utilizing the homology vector 807-41.03 and virus S-SPV-122 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of red plaque purification was the recombinant virus designated S-SPV-158. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

To confirm the expression of the SIV HA, NA, and NP gene products, cells were infected with S-SPV-158 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal goat

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anti-HA, a polyclonal goat anti-NP, or a polyclonal goat anti-NA serum was used to detect expression of SIV specific proteins. The cell lysate from SPV-158 infected cells exhibited bands corresponding to 64 kd, 52 kd, and 56 kd, which is the expected size of the SIV HA, NA, and NP proteins, respectively.

S-SPV-158 was assayed for expression of SIV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. A polyclonal swine anti-SIVserum, a polyclonal goat anti-HA serum, or a polyclonal goat anti-NP serum was shown to react specifically with S-SPV-158 plaques and not with S-SPV-001 negative control plaques. All S-SPV-158 observed plaques reacted with all three serological reagents indicating that the virus was stably expressing the SIV foreign genes. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines. --

S-SPV-158 is a recombinant swinepox virus expressing the SIV HA, NA, and NP proteins and is useful as a vaccine in swine against SIV infection. S-SPV-158 is also useful for expression of the SIV HA, NA, and NP proteins.

HOMOLOGY VECTOR 807-41.03. The homology vector 807-41.03 was used to insert foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the swine influenza virus (SIV) nucleoprotein (NP) gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter

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(LP1) and the SIV NP gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining
5 restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base
10 pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 2 is an approximately 1501 base pair EcoRI to EcoRI fragment of the SIV NP gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from
15 the SIV H1N1 strain (NVSL). The upstream primer (5'-CATGAATTCTCAAGGCACCAAACGATCATATGAAC-3') synthesizes from the 5' end of the SIV NP gene and introduces an EcoRI site at the 5' end of the gene. The downstream primer was (5'-ATTTGAATTCAATTGTCATACTCCTCTGCATTGTCT-3')
20 synthesizes from the 3' end of the SIV NP gene, introduces an EcoRI site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI to yield a fragment 1501 base pairs in length
25 corresponding to the SIV NP gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction
30 fragment M (23). The AccI site in the SPV homology vector was converted to a unique NotI site using synthetic linkers.

35 S-SPV-217:

S-SPV-217 is a swinepox virus that expresses four foreign genes. The gene for porcine reproductive and

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respiratory syndrome virus (PRRS) ORF5 and the gene for E. coli β -galactosidase (lacZ) are inserted into a unique Not I restriction site (NotI linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF6 and the gene for E. coli β -glucuronidase (uidA) are inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The porcine reproductive and respiratory syndrome virus (PRRS) ORF6 gene is under the control of the synthetic early promoter (EP1), the porcine reproductive and respiratory syndrome virus (PRRS) ORF5 gene is under the control of the synthetic early promoter (EP2). The lacZ gene is under the control of the synthetic late promoter (LP1), the uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-217 is derived from S-SPV-174 (Kasza Strain). This was accomplished utilizing a homology vector and virus S-SPV-174 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -glucuronidase (SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of blue plaque purification is the recombinant virus designated S-SP-217. This virus is assayed for β -galactosidase and β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods.

S-SPV-217 is a recombinant swinepox virus expressing the PRRS ORF5 and ORF6 proteins and is useful as a vaccine in swine against PRRS infection. S-SPV-134 is

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also useful for expression of the PRRS ORF5 and ORF6 proteins.

S-SPV-218:

5 S-SPV-218 is a swinepox virus that expresses four foreign genes. The gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF5 and the gene for E. coli β -galactosidase (lacZ) are inserted into a unique Not I restriction site (NotI linkers inserted
10 into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF6 and the gene for E. coli β -glucuronidase (uidA) are inserted into a unique NotI site (NotI linkers inserted
15 into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The porcine reproductive and respiratory syndrome virus (PRRS) ORF6 gene is under the control of the synthetic late promoter (LP1), the porcine reproductive and respiratory syndrome virus (PRRS) ORF5
20 gene is under the control of the synthetic early promoter (EP2). The lacZ gene is under the control of the synthetic late promoter (LP1), the uidA gene is under the control of the synthetic early promoter
25 (EP2).

S-SPV-218 is derived from S-SPV-174 (Kasza Strain). This was accomplished utilizing a homology vector and virus S-SPV-174 in the HOMOLOGOUS RECOMBINATION
30 PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -glucuronidase (SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of blue plaque purification is
35 the recombinant virus designated S-SP-218. This virus is assayed for β -galactosidase and β -glucuronidase expression, purity, and insert stability by multiple

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passages monitored by the blue plaque assay as described in Materials and Methods.

5 S-SPV-218 is a recombinant swinepox virus expressing the PRRS ORF5 and ORF6 proteins and is useful as a vaccine in swine against PRRS infection. S-SPV-134 is also useful for expression of the PRRS ORF5 and ORF6 proteins.

10

S-SPV-195:

15 S-SPV-195 is a swinepox virus that expresses one foreign gene. The full length gene encoding the surface and transmembrane protein subunits for FeLV (SU+TM) and the gene for E. coli β -galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The FeLV envelope gene is under the control of the synthetic early promoter (EP1) and the lacZ gene is under the control of the synthetic late promoter (LP1).

20 S-SPV-195 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 911-4.A1 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase. (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of red plaque purification was the recombinant virus designated S-SPV 195. This virus was assayed for β -galactosidase expression and is currently under going multiple passages to determine purity and stability.

35

S-SPV-195 was assayed for expression of FeLV envelope, gp70, using the BLACK PLAQUE SCREEN FOR FOREIGN GENE

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5 EXPRESSION IN RECOMBINANT SPV. Monoclonal mouse anti-FeLV gp70 was shown to react specifically with S-SPV-195 plaques and not with S-SPV-001 negative control plaques. All S-SPV-195 plaques observed reacted with the monoclonal antibody indicating that the virus was stably expressing the FeLV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

10 S-SPV-195 is a recombinant swinepox virus expressing the FeLV envelope proteins and is useful as a vaccine in cats against feline leukemia infection. S-SPV-195 is also useful for expression of the FeLV envelope protein.

20 HOMOLOGY VECTOR 911-4.A1. The homology vector 911-4.A1 was used to insert foreign DNA into SPV. It incorporates an E. coli β -galactosidase (*lacZ*) marker gene and the full-length FeLV envelope gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA

25 coding for the foreign genes results. Note that the β galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter (LP1) and the FeLV envelope gene is under the control of a synthetic early pox promoter (EP1). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair

30 HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV

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HindIII fragment M (23). Fragment 2 is an approximately 1929 base pair EcoRI to BamHI fragment of the FeLV envelope gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using FeLV cDNA from the p61E, subtype A FeLV/FAIDs strain. The upstream primer (5'-CGTCGGATCCGGACAGCCCCAGCTTAGACGATC-3') synthesizes from the 5' end of the FeLV envelope gene and introduces an EcoRI site at the 5' end of the gene. The downstream primer (5'-CGTCGGATCCGGGGACTAAATGGAATCATAACA-3') synthesizes from the 3' end of the FeLV envelope gene, and introduces a BamHI site at the 3' end of the gene. These primers were used for reverse transcription and polymerase chain reaction by CLONING WITH THE POLYMERASE CHAIN REACTION. The PCR product was digested with EcoRI and BamHI to yield a fragment of approximately 1929 base pairs in length corresponding to the FeLV envelope gene.

20

Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology vector was converted to a unique Not I site using synthetic linkers.

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S-SPV-205:

30 S-SPV-205 is a swinepox virus that expresses four foreign genes. The gene for feline leukemia virus (FeLV) gag/protease the gene for E. coli β -galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for FeLV envelope and β -glucuronidase (uidA) were inserted into a unique Not

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I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The FeLV gag/protease gene is under the control of the synthetic late/early promoter (LP2EP2), and the FeLV envelope gene is under the control of a synthetic early promoter (EP1). The lacZ gene is under the control of the synthetic late promoter, LP1 and the uidA gene is under the control of the synthetic early pox promoter, EP2.

10

S-SPV-205 was derived from S-SPV-089 (Kasza Strain). This was accomplished by utilizing the homology vector 905-31.A1 and virus S-SPV-089 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -glucuronidase (X-GLUC and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). Recombinant plaques expressing the marker gene were shown to be positive for β -glucuronidase by blue/green plaque detection and were designated as SPV 205.

15

This virus was assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue/green plaque assay as described in Materials and Methods. After the initial three rounds of passage, all plaques observed are blue/green, indicating that the virus is pure. Analysis of expression of the FeLV gag and envelope genes and stability analysis are in progress.

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30

HOMOLOGY VECTOR 905-31.A1. The homology vector 905-31.A1 was used to insert foreign DNA into SPV089. It incorporates an E. coli β -glucuronidase (uidA) marker gene and the FeLV envelope gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING

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RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β -glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2) and the FeLV envelope gene is under the control of a separate and unique synthetic early pox promoter (EP1). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 905-31.A1 was constructed using plasmid 847-42.2C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The FeLV envelope gene was then inserted into a unique Not I site located upstream of the uidA gene resulting in plasmid 905-31.A1. The FeLV envelope gene is an approximately 1929 base pair EcoRI to BamHI fragment synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using FeLV cDNA from the p61E, subtype A FeLV/FAIDs strain. The upstream primer (5'-CGTCGGATCCGGACAGCCCCAGCTTAGACGATC-3') synthesizes from the 5' end of the FeLV envelope gene and introduces an EcoRI site at the 5' end of the gene. The downstream primer (5'-CGTCGGATCCGGGGACTAAATGGAATCATACA-3') synthesizes from the 3' end of the FeLV envelope gene, and introduces a BamHI site at the 3' end of the gene. These primers were used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI and BamHI to yield a fragment of approximately 1929 base pairs in length corresponding to the FeLV envelope gene.

35 S-SPV-197;

S-SPV-197 is a swinepox virus that expresses one foreign gene. The full-length envelope gene from feline

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leukemia virus, subtype A (FeLV) and the gene encoding E. coli β -glucuronidase (uidA) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for FeLV envelope was inserted into a unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The envelope gene is under the control of the synthetic early promoter (EP2) and the uidA gene is under the control of a separate and unique synthetic early promoter (EP2).

S-SPV-197 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 905-31.A1 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -glucoronidase (X-GLUC and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of blue/green purification was the recombinant virus designated S-SPV-197.

This virus was assayed for β -glucoronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue/green indicating that the virus is pure, stable, and expressing the foreign genes.

To confirm the expression of the FeLV envelope foreign gene product, cells were infected with S-SPV-197 and samples of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A

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mouse monoclonal anti-FelVgp70 antibody was used to detect expression of FeLV gp70 specific protein. The cell lysate from SPV-195 infected cells exhibited bands corresponding to 85kda and 70kda, which are the expected sizes for the unprocessed FeLV envelope protein and the processed gp 70 envelope surface protein. The 70kda protein product was predominant in the cell lysates.

5 S-SPV-197 was assayed for expression of FeLV envelope, gp70, using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Monoclonal mouse anti-FeLV gp70 was shown to react specifically with S-SPV-197 plaques and not with S-SPV-001 negative control plaques. All S-SPV-197 plaques observed reacted with the monoclonal antibody indicating that the virus was stably expressing the FeLV envelope gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-197 is a recombinant swinepox virus expressing the FeLV envelope proteins and is useful as a vaccine in cats against feline leukemia infection. S-SPV-197 is also useful for expression of the FeLV envelope protein.

HOMOLOGY VECTOR 905-31.A1: The homology vector 905-31.A1 was used to insert foreign DNA into SPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene and the FeLV envelope gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes results. Note that the β -glucuronidase (uidA) marker gene is under the control of a synthetic

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early pox promoter (EP2) and the FeLV envelope virus gene is under the control of a separate and unique synthetic early pox promoter (EP1). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 905-31.A1 was constructed using plasmid 847-42.2C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The FeLV envelope gene was then inserted into a unique Not I site located upstream of the uidA gene resulting in plasmid 905-31.A1.

15 The FeLV envelope gene is an approximately 1929 base pair EcoRI to BamHI fragment synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using FeLV cDNA from the p61E, subtype A FeLV/FAIDs strain. The upstream primer (5'-CGTCGGATCCGGACAGCCCCAGCTTAGACGATC-3') synthesizes from the 5' end of the FeLV envelope gene and introduces an EcoRI site at the 5' end of the gene. The downstream primer (5'-CGTCGGATCCGGGGACTAAATGGAATCATACA-3') synthesizes from the 3' end of the FeLV envelope gene, and introduces a BamHI site at the 3' end of the gene. These primers were used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI and BamHI to yield a fragment of approximately 1929 base pairs in length corresponding to the FeLV envelope gene.

S-SPV-198:

35 S-SPV-198 is a swinepox virus that expresses two foreign genes. The full-length envelope gene from feline leukemia virus, subtype A (FeLV) and the gene

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encoding *E. coli* β -glucuronidase (*uidA*) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique *AccI* restriction site in the *O1L* ORF of the SPV HindIII M fragment). The gene for FeLV envelope was inserted into a unique Not I site (Not I linkers inserted into a unique *EcoRI* restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The β -glucuronidase gene is under the control of the synthetic early pox promoter, EP2.

10 The FeLV envelope gene is under the control of the early/late synthetic pox promoter, EP1. Note that the two promoter/gene cassettes are orientated in opposing directions, to avoid possible homologous recombination between identical promoter elements (EP2).

15 S-SPV-198 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 860-2.A5 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV.

20 The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -glucoronidase (X-GLUC and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). Recombinant plaques expressing the marker gene were shown to be positive for

25 β -glucuronidase by blue/green plaque detection and are designated as SPV 198.

S-SPV-198 is in the process of purification, analysis of foreign gene expression and stability using black

30 plaque and western blot assays, as described in Materials and Methods. The assays described here are carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

35 S-SPV-198 is a recombinant swinepox virus expressing the FeLV envelope protein and is useful as a vaccine in

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cats against feline leukemia infection. S-SPV-198 is also useful for expression of the FeLV envelope protein.

5 HOMOLOGY VECTOR 860-2.A5. The homology vector 860-2.A5 was used to insert foreign DNA into SPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene and the FeLV envelope gene flanked by SPV DNA. When this homology vector was used according to the
10 HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes results. Note that the β -glucuronidase (uidA) marker gene is under the early synthetic pox promoter (EP2) and the FeLV envelope gene is under the
15 control of a synthetic early/late pox promoter (LP2/EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA
20 sequences. Plasmid 860-2.A5 was constructed using plasmid 847-42.2C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The FeLV envelope gene was then inserted into a unique
25 NotI site located upstream of the uidA gene resulting in plasmid 860-2.A5.

The FeLV envelope gene is an approximately 1929 base pair EcoRI to BamHI fragment synthesized by reverse
30 transcription (RT) and polymerase chain reaction (PCR) (15,42) using FeLV cDNA from the p61E, subtype A, FeLV/FAIDs strain (received from the NIAIDS repository, cat. # 109). The upstream primer
(5'-CGTCGGATCCGGACAGCCCCAGCTTAGACGATC-3') synthesizes
35 from the 5' end of the FeLV envelope gene and introduces an EcoRI site at the 5' end of the gene. The downstream primer

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introduces an EcoRI site at the 5' end of the gene.

The downstream primer

(5'-CGTCGGATCCGGGGACTAAATGGAATCATAACA-3') synthesizes
from the 3' end of the FeLV envelope gene, and

5 introduces a BamHI site at the 3' end of the gene.

These primers were used for reverse transcription and
polymerase chain reaction. The PCR product was

digested with EcoRI and BamHI to yield a fragment of
approximately 1929 base pairs in length corresponding

10 to the FeLV envelope gene.

S-SPV-206:

S-SPV-206 is a swinepox virus that expresses four
foreign genes. The gene for FIV envelope and the gene

15 for E. coli β -galactosidase (lacZ) were inserted into
a unique Not I restriction site (Not I linkers inserted
into a unique AccI restriction site in the O1L ORF of
the SPV HindIII M fragment). The gene for FIV
gag/protease and the gene for E.coli

20 β -glucuronidase(uidA) was inserted into a unique Not I
site (Not I linkers inserted into a unique EcoRI
restriction site within an approximately 3.2 kb region
of the 6.7 kb SPV HindIII K fragment). The FIV
gag/protease and envelope genes are under the control
25 of separate, and identical synthetic late/early
promoters (LP2EP2). The lacZ gene is under the control
of the synthetic late promoter (LP1) and the uidA gene
is under the control of the synthetic early promoter
(EP2).

30

S-SPV-206 was derived from S-SPV-048. This was
accomplished utilizing the homology vector 913-11.4 and
virus S-SPV-048 in the HOMOLOGOUS RECOMBINATION
PROCEDURE FOR GENERATING RECOMBINANT SPV. The
35 transfection stock was screened by the SCREEN FOR
RECOMBINANT SPV EXPRESSING β -glucuronidase (X-GLUC
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EXPRESSING ENZYMATIC MARKER GENES). The final result of blue/green purification was the recombinant virus designated S-SPV-206. This virus was assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue/green plaque assay as described in Materials and Methods. After the initial three rounds of passage, all plaques observed were blue/green indicating that the virus is pure, stable, and expressing the foreign genes.

To confirm the expression of the FIV gag/protease and envelope gene products, cells were infected with S-SPV-206 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal cat anti-FIV PPR serum or a monoclonal mouse anti-FIVgag(P24) antibody was used to detect expression of FIV envelope and gag specific proteins. The cell lysate from SPV-200 infected cells exhibited bands corresponding expected size bands of the FIV gag/protease and envelope proteins.

S-SPV-200 was assayed for expression of FIV gag/protease specific antigen using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Monoclonal mouse anti-FIV gag (P24) antibody was shown to react specifically with S-SPV-206 plaques and not with S-SPV-001 negative control plaques. All S-SPV-206 observed plaques reacted with this antibody indicating that the virus was stably expressing the FIV gag/protease foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

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S-SPV-206 is a recombinant swinepox virus expressing the FIV gag/protease and envelope proteins and is useful as a vaccine in cats against FIV infection. S-SPV-206 is also useful for expression of the FIV gag/protease and envelope genes.

HOMOLOGY VECTOR 913-11.4. The homology vector 913-11.4 was used to insert foreign DNA into SPV 048. It incorporates an E. coli β -glucuronidase (uidA) marker gene and FIV gag/protease gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the uidA marker gene is under the control of a synthetic early pox promoter EP2 and the FIVgag/protease is under the control of a synthetic late/early pox promoter (LP2EP2). These two promoter/gene cassettes were oriented in opposing directions with the promoters adjacent, but in opposite orientations to avoid homologous recombination between identical EP2 promoter elements. The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 913-11.4 was constructed using plasmid 847-42.2C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter (EP2). The FIVgag/protease gene was then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 913-11.4.

The FIV gag/protease gene was synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using cDNA from the FIV PPR strain. The

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u p s t r e a m p r i m e r
(5'-GCGTGAATTCGGGGAATGGACAGGGGCGAGAT-3') synthesizes
from the 5' end of the FIV gag/protease gene and
introduces an EcoRI site at the 5' end of the gene. The
5 downstream primer was (5'-GAGCCAGATCTGCTCTTTTACTTTCCC
-3') synthesizes from the 3' end of the FIV
gag/protease gene, introduces an BglII site at the 3'
end of the gene, and was used for reverse transcription
and polymerase chain reaction. The PCR product was
10 digested with EcoRI and BglII to yield a fragment
approximately 1839 base pairs in length corresponding
to the FIV gag/protease gene.

S-SPV-200:

15 S-SPV-200 is a swinepox virus that expresses three
foreign genes. The genes for feline immunodeficiency
virus (FIV) gag/protease, and full length envelope and
the gene for E. coli β -galactosidase (lacZ) were
inserted into a unique Not I restriction site (Not I
20 linkers inserted into a unique AccI restriction site in
the O1L ORF of the SPV HindIII M fragment). The
FIVgag/protease and envelope genes are under the
control of separate, but identical synthetic late/early
promoter (LP2EP2. The lacZ gene is under the control
25 of the synthetic late promoter (LP1).

S-SPV-200 was derived from S-SPV-001 (Kasza Strain).
This was accomplished utilizing the homology vector
904-63.B7 and virus S-SPV-001 in the HOMOLOGOUS
30 RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV.
The transfection stock was screened by the SCREEN FOR
RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND
CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS
EXPRESSING ENZYMATIC MARKER GENES). The final result of
35 red plaque purification was the recombinant virus
designated S-SPV-157. This virus was assayed for
 β -galactosidase expression by the blue plaque assay as

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described in Materials and Methods. Analysis of purity, and insert stability by multiple passages is in progress and will be monitored by the blue plaque assay as described in Materials and Methods.

5

S-SPV-200 is a recombinant swinepox virus expressing the FIVgag/protease and FIV envelope proteins and is useful as a vaccine in swine against SIV infection. S-SPV-200 is also useful for expression of the FIV
10 gag/protease and envelope proteins.

HOMOLOGY VECTOR 904-63.B7. The homology vector 904-63.B7 was used to insert foreign DNA into SPV 001. It incorporates an E. coli β -galactosidase (lacZ)
15 marker gene and the feline immunodeficiency virus (FIV) gag/protease and envelope genes flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the
20 foreign genes results. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the FIV gag/protease and envelope genes are under the control of separate, but identical synthetic late/early pox promoters (LP2EP2).
25 The FIVgag/protease and FIV envelope promoter/gene cassettes are oriented in opposing directions such that transcription of the gag/protease and envelope genes runs toward each other to avoid the possibility of homologous recombination between identical promoters.
30 The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair
35 HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV

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HindIII fragment M (23). Fragment 2 is an approximately 2580 base pair EcoRI to BglII fragment of the FIV envelope gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using cDNA from the FIV PPR strain. The upstream primer (5'-GCCCCGATCCTATGGCAGAAGGGTTTGCAGC-3') synthesizes from the 5' end of the FIV envelope gene and introduces a BamHI site at the 5' end of the gene. The downstream primer was (5'-CCGTGGATCCGGCACTCCATCATTCCTCCTC -3') synthesizes from the 3' end of the FIV envelope gene, introduces an BamHI site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with BamHI to yield a fragment 2580 base pairs in length corresponding to the FIV envelope gene. Fragment 3 is an approximately 1839 base pair EcoRI to BglII fragment of the FIV gag/protease gene synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using cDNA from the FIV PPR strain. The upstream primer (5'-GCGTGAATTCGGGGAATGGACAGGGGCGAGAT-3') synthesizes from the 5' end of the FIV gag/protease gene and introduces an EcoRI site at the 5' end of the gene. The downstream primer was (5'-GAGCCAGATCTGCTCTTTTACTTTCCC -3') synthesizes from the 3' end of the FIV gag/protease gene, introduces an BglII site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with EcoRI and BglII to yield a fragment approximately 1839 base pairs in length corresponding to the FIV gag/protease gene. Fragment 4 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 5 is an approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology

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vector was converted to a unique NotI site using synthetic linkers.

S-SPV-207:

5 S-SPV-207 is a swinepox virus that expresses four foreign genes. The gene for FIV gag/protease and the gene for E. coli β -galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique AccI restriction site in the O1L
10 ORF of the SPV HindIII M fragment). The gene for FIV envelope and the gene for E.coli β -glucuronidase(uidA) was inserted into a unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII
15 K fragment). The FIV gag/protease gene is under the control of the late/early promoters (LP2EP2). The lacZ gene is under the control of the constitutive SPV promoter, O1L. The FIV envelope gene is under the control of the synthetic early pox promoter (EP1) and
20 the uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-207 was derived from S-SPV-046. This was accomplished utilizing the homology vector 911-96.A2
25 and virus S-SPV-046 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -glucuronidase (X-GLUC ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS
30 EXPRESSING ENZYMATIC MARKER GENES). The final result of blue/green purification was the recombinant virus designated S-SPV-207. Initial virus purifications were assayed for β -glucuronidase expression by the blue/green plaque assay as described in Materials and
35 Methods. Final purification and expression analysis of foreign genes is in progress.

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S-SPV-207 is a recombinant swinepox virus expressing the FIV gag/protease and envelope proteins and is useful as a vaccine in cats against FIV infection. S-SPV-207 is also useful for expression of the FIV
5 gag/protease and envelope genes.

HOMOLOGY VECTOR 911-96.A2. The homology vector 911-96.A2 was used to insert foreign DNA into SPV 046. It incorporates an E. coli β -glucuronidase (uidA)
10 marker gene and FIV envelope gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the uidA marker gene
15 is under the control of a synthetic early pox promoter (EP2) and the FIV envelope is under the control of a synthetic early pox promoter (EP1). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction
20 fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 911-96.A2 was constructed using plasmid 847-42.2C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K
25 genomic fragment. The uidA gene is under the control of the synthetic early promoter (EP2). The FIV envelope gene was then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 911-96.A2.

30

The FIV envelope gene was synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using cDNA from the FIV PPR strain. The upstream primer (5'-GCCCGGATCCTATGGCAGAAGGGTTTGCAGC
35 -3') synthesizes from the 5' end of the FIV envelope gene and introduces a BamHI site at the 5' end of the gene. The downstream primer was

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was used for reverse transcription and polymerase chain reaction. The PCR product was digested with BamHI to yield a fragment approximately 2580 base pairs in length corresponding to the FIV gag/protease gene.

5

S-SPV-142:

S-SPV-142 is a swinepox virus that expresses three foreign genes. The genes for bovine viral diarrhea virus type 1 (BVDV1) E2 glycoprotein and the gene for
10 E. coli β -galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The genes for bovine viral diarrhea virus type 1 (BVDV1) E^{ns} glycoprotein, bovine
15 viral diarrhea virus type 2 (BVDV2) E2 glycoprotein and the E. coli β -glucuronidase (uidA) marker gene were inserted into unique Bam HI, Not I and Pst I sites respectively (sites originating from a synthetic polylinker linker inserted into a unique EcoRI
20 restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The E2 genes are under the control of the synthetic late/early promoter (LP2EP2), The E^{ns} gene and the lacZ gene are under the control of the synthetic late promoter (LP1), and the
25 uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-142 was derived from S-SPV-051 (Kasza Strain). This was accomplished utilizing the homology vector
30 874-4.6A and virus S-SPV-051 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The BVDV type 1 E2 gene and the lacZ gene in the Hind III M site were already present in virus S-SPV-051. The transfection stock was screened by the SCREEN FOR
35 RECOMBINANT SPV EXPRESSING β -glucuronidase (X-Gluc ASSAY and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of green

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plaque purification was the recombinant virus designated S-SPV-142. This virus was assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the green
5 plaque assay as described in Materials and Methods. After the initial four rounds of purification, all plaques observed are green indicating that the virus is pure, stable, and expressing the foreign genes.

10 To confirm the expression of the BVDV type 2 E2 and type 1 E^{ns} gene products, cells were infected with S-SPV-142 and samples of infected cell lysates and culture supernatants were subjected to SDS
15 polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A monoclonal mouse anti-E2 (type 2) or a monoclonal mouse anti-E^{ns} (type 1) serum was used to detect expression of BVDV-specific proteins. The cell lysate from
20 S-SPV-142 infected cells exhibited bands corresponding to 53 kd and 42 kd, which is the expected size of the E2 glycoprotein (53 kd) but slightly smaller than the expected size of the E^{ns} glycoprotein (48 kd).

S-SPV-142 was assayed for expression of BVDV-specific
25 antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Monoclonal mouse anti-E2 sera were shown to react specifically with S-SPV-142 plaques and not with S-SPV-001 negative control
30 plaques. All S-SPV-142 observed plaques reacted with both a type 1 E2 and a type 2 E2 serum indicating that the virus was stably expressing the BVDV E2 foreign genes. No reagent is currently available that reacts specifically with the E^{ns} glycoprotein in a black
35 plaque assay. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

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CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of blue plaque purification was the recombinant virus designated S-SPV 187. This virus was assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial 5 rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

S-SPV-187 was assayed for expression of NDV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Monoclonal mouse anti-NDV F was shown to react specifically with S-SPV-187 plaques and not with S-SPV-003 negative control plaques. All S-SPV-187 observed plaques reacted with the monoclonal antibody indicating that the virus was stably expressing the NDV foreign genes. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-187 is a recombinant swinepox virus expressing the NDV F protein and is useful as a vaccine in chickens against NDV infection. S-SPV-187 is also useful for expression of the NDV F protein.

059 HOMOLOGY VECTOR CONTAINING FOREIGN GENE AND UDA: HOMOLOGY VECTOR 894-21.25 . The homology vector 894-21.25 was used to insert foreign DNA into SPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene and the NDV F gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the

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results. Note that the β -glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2) and the NDV F gene is under the control of a synthetic early/late pox promoter (EP1/LP2). The
5 homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 894-21.22 was constructed using plasmid 847-42.7C and
10 plasmid 493-91.11. Plasmid 847-42.7C was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter (EP2). Plasmid 493-11.1 was previously
15 constructed and contains the EP1/LP2 promoter upstream of the coding region of the NDV F gene, an approximately 2100 base pair fragment derived from the full length molecular clone (5025F). The EP1/LP2-NDV F cassette was then inserted into a unique NotI site
20 located upstream of the uidA gene resulting in plasmid 894-21.25. The transcriptional and translational orientation of the NDV F gene is opposite to that of the uidA gene.

25 **S-SPV-188:**

S-SPV-188 is a swinepox virus that expresses 4 foreign genes. The gene for Newcastle disease virus (NDV) HN and the gene for E. coli β -galactosidase (lacZ) were inserted into a unique NotI restriction site (NotI
30 linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for Newcastle disease virus (NDV) F and the gene for E. coli β -glucuronidase (uidA) were inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI
35 restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The NDV HN gene is under the control of the synthetic early/late

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promoter (EP1/LP2), the NDV F gene is under the control of the synthetic early/late promoter (EP1/LP2). The lacZ gene is under the control of the synthetic late promoter (LP1), the uidA gene is under the control of the synthetic early promoter (EP2).

S-SPV-188 was derived from S-SPV-009 (Kasza Strain), which contains the EP1/LP2-NDV HN and LP1-LacZ in the 003 site. This was accomplished utilizing the homology vector 894-21.25 and virus S-SPV-009 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -glucuronidase (BLUOGAL AND CPRG ASSAYS and SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of blue plaque purification was the recombinant virus designated S-SPV 188. This virus was assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial five rounds of purification, all plaques observed were blue indicating that the virus is pure, stable, and expressing the foreign genes.

S-SPV-188 was assayed for expression of NDV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Monoclonal mouse anti-NDV F and monoclonal mouse anti-NDV HN were shown to react specifically with S-SPV-188 plaques and not with S-SPV-003 negative control plaques. All S-SPV-188 observed plaques reacted with the monoclonal antibodies indicating that the virus was stably expressing the NDV foreign genes. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

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EXPRESSION IN RECOMBINANT SPV. Monoclonal mouse anti-NDV F and monoclonal mouse anti-NDV HN were shown to react specifically with S-SPV-188 plaques and not with S-SPV-003 negative control plaques. All S-SPV-188
5 observed plaques reacted with the monoclonal antibodies indicating that the virus was stably expressing the NDV foreign genes. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV
10 recombinant vaccines.

S-SPV-188 is a recombinant swinepox virus expressing the NDV HN and F proteins and is useful as a vaccine in chickens against NDV infection. S-SPV-188 is also
15 useful for expression of the NDV HN and F proteins.

059 HOMOLOGY VECTOR CONTAINING FOREIGN GENE AND UIDA:
HOMOLOGY VECTOR 894-21.25 . The homology vector 894-21.25 was used to insert foreign DNA into SPV. It
20 incorporates an E. coli β -glucuronidase (uidA) marker gene and the NDV F gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes
25 results. Note that the β -glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2) and the NDV F gene is under the control of a synthetic early/late pox promoter (EP1/LP2). The homology vector was constructed utilizing standard
30 recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 894-21.22 was constructed using plasmid 847-42.7C and plasmid 493-91.11. Plasmid 847-42.7C was previously
35 constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic

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early promoter (EP2). Plasmid 493-11.1 was previously constructed and contains the EP1/LP2 promoter upstream of the coding region of the NDV F gene, an approximately 2100 base pair fragment derived from the full length molecular clone (5025F). The EP1/LP2-NDV F cassette was then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 894-21.25. The transcriptional and translational orientation of the NDV F gene is opposite to that of the uidA gene.

S-SPV-148:

S-SPV-148 is a swinepox virus that expresses two foreign genes. The gene for bovine herpesvirus (BHV-1) truncated glycoprotein D (gD) and the gene for E. coli β -galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gD gene is under the control of the synthetic late/early promoter (LP2EP2) and the lacZ gene is under the control of the synthetic late promoter (LP1).

S-SPV-148 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 859-52.30 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-148. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are

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blue indicating that the virus is pure, stable, and expressing the foreign genes.

To confirm the expression of the BHV-1 gD gene product, cells were infected with S-SPV-148 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal bovine anti-BHV-1 serum was used to detect expression of BHV-1 specific proteins. The cell lysate from SPV-148 infected cells exhibited bands corresponding to 60 kd, which is the expected size of the BHV-1 truncated gD protein.

S-SPV-148 is a recombinant swinepox virus expressing the BHV-1 gD protein and is useful as a vaccine in cows against BHV-1 infection. S-SPV-148 is also useful for expression of the BHV-1 gD protein. Infectious bovine rhinotracheitis virus glycoprotein D (IBR gD) having a deleted transmembrane domain improves the immune response against IBR gD and improves the vaccine efficiency.

HOMOLOGY VECTOR 859-52.30. The homology vector 859-52.30 was used to insert foreign DNA into SPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and the truncated bovine herpesvirus glycoprotein D (gD) gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the truncated BHV-1 gD gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by

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joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector was derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglII to AccI restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 2 is an approximately 1086 base pair EcoRI to BamHI fragment of the BHV-1 truncated gD gene synthesized by polymerase chain reaction (PCR) (15,42) using DNA from the BHV-1 Cooper strain genomic fragment. The upstream primer (5' - CGGAATTCACAAGGGCCGACATTGGCC -3') synthesizes from the 5' end of the BHV-1 gD gene and introduces an EcoRI site at the 5' end of the gene. The downstream primer was (5' - GCTGGGATCCACGGCGTCGGGGGCCGCGGGCGT -3') synthesizes from the 3' end of the BHV-1 gD gene, introduces an BamHI site at the 3' end of the gene. The PCR product was digested with EcoRI and BamHI to yield a fragment 1086 base pairs in length corresponding to the BHV-1 truncated gD gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII restriction sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology vector was converted to a unique NotI site using synthetic linkers.

30 S-SPV-186:

S-SPV-186 is a swinepox virus that expresses two foreign genes. The gene for canine distemper virus (CDV) hemagglutinin (HA) and the gene and the gene for E. coli E. coli β -glucuronidase (uidA) were inserted into a unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment).

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The HA gene is under the control of the synthetic late promoter (LP2), and the uidA gene is under the control of the synthetic early promoter (EP2).

5 S-SPV-186 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 899-20 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR
10 RECOMBINANT SPV EXPRESSING β -glucuronidase (x-gluc). The final result of red plaque purification was the recombinant virus designated S-SPV-186. This virus was assayed for β glucuronidase expression, purity, and insert stability by multiple passages monitored by the
15 blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

20 To confirm the expression of the CDV HA gene product, cells were infected with S-SPV-186 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis.
25 The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal dog anti-CDV serum was used to detect expression of CDV specific protein. The cell lysate from SPV-186 infected cells exhibited bands corresponding to 70kd, which is the expected size of
30 the CDV HA protein.

S-SPV-186 is a recombinant swinepox virus expressing the CDV HA protein and is useful as a vaccine in dogs against CDV infection. S-SPV-186 is also useful for
35 expression of the CDV HA protein.

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HOMOLOGY VECTOR 899-20. The homology vector 899-20 was used to insert foreign DNA into SPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene and the canine distemper virus (CDV) hemagglutinin (HA) gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β -glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2) and the CDV HA gene is under the control of a synthetic late pox promoter (LP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), The CDV HA gene is an approximately 1875 base pair BglII fragment synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the CDV (NVSL challenge strain). The upstream primer (5' - GAAGATCTAATGCTCTCCTACCAAGACAAGGTGGGTGCCT-3') synthesizes from the 5' end of the CDV HA gene and introduces an BglII site at the 5' end of the gene. The downstream primer was (5' - GAAGATCTTCAAGGTTTGAACGGTCACATGAGAATCTT -3') synthesizes from the 3' end of the CDV HA gene, introduces an BglII site at the 3' end of the gene, and was used for reverse transcription and polymerase chain reaction. The PCR product was digested with BglII to yield a fragment 1875 base pairs in length corresponding to the CDV HA gene.

30

S-SPV-185:

S-SPV-185 is a swinepox virus that expresses five foreign genes. The gene for bovine herpesvirus BHV-1 glycoprotein D (gD) and the gene for E. coli β -galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a

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unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for bovine cytokine interleukin-12 (bIL-12) p40, p35 and the gene for E. coli β -glucuronidase (uidA) genes were inserted into a

5 unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The gD gene is under the control of the synthetic late/early promoter (LP2EP2). The bIL-12 p40 and p35 genes are

10 under the control of the synthetic late promoter (LP2 and LP1), the uidA gene is under the control of synthetic early promoter (EP2) and the lacZ gene is under the control of the synthetic late promoter (LP1).

15 S-SPV-185 was derived from S-SPV-148 (Kasza Strain). This was accomplished utilizing the homology vector 870-56 and virus S-SPV-148 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR

20 RECOMBINANT SPV EXPRESSING β -glucuronidase (x-gluc). The final result of red plaque purification was the recombinant virus designated S-SPV-185. This virus was assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the

25 blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

30 To confirm the expression of the BHV-1 gD and bIL-12 p40, p35 gene products, cells were infected with S-SPV-185 and samples of infected cell lysates and culture supernatants were subjected to SDS

35 polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal goat anti-hIL-12 and polyclonal bovine

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anti-BHV sera were used to detect expression of bIL-12 and BHV-1 gD specific proteins. The cell lysate from SPV-185 infected cells exhibited bands corresponding to 60 kd, 40kd and 35 kd, which is the expected size of the BHV-1 truncated gD, bIL-12 p40 and p35 proteins.

S-SPV-185 was assayed for expression of bIL-12 specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal goat anti-bIL-12 serum was shown to react specifically with S-SPV-185 plaques and not with S-SPV-148 negative control plaques. All S-SPV-185 observed plaques reacted with serological reagent indicating that the virus was stably expressing the bovine foreign genes. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-185 is a recombinant swinepox virus expressing the bovine antigen proteins and is useful as a vaccine in cows against viral infection. S-SPV-185 is also useful for expression of the BHV-1 gD, bIL-12 p40 and p35 proteins. A vaccine containing S-SPV-185 stimulates cell mediated immunity and improves growth and weight gain of the animal.

HOMOLOGY VECTOR 870-56. The homology vector 870-56 was used to insert foreign DNA into SPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene and the bovine cytokine interleukin-12 (bIL-12) p40 and p35 genes flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β -glucuronidase (uidA) marker gene is under the control of a synthetic early pox

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promoter (EP2) and bovine cytokine interleukin-12 genes (bIL-12) is under the control of a synthetic late pox promoters (LP2 and LP1). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 870-56 was constructed using plasmid 847-42.2C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter (EP2). The bIL-12 genes were then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 870-56. The transcriptional and translational orientation of the bIL-12 genes are the same as the uidA gene.

The bIL-12 p40 gene is an approximately 984 base pair BamHI to BamHI fragment synthesized by polymerase chain reaction (PCR) (15,42) using DNA from the plasmid containing IL-12 gene. The upstream primer (5'-CGTCGGATCCAATGCACCCTCAGCAGTTGGTC-3') synthesizes from the 5' end of the bIL-12 p40 gene and introduces an BamHI site at the 5' end of the gene. The downstream primer was (5'-GTTGGATCCTAACTGCAGGACACAGATGCCC-3') synthesizes from the 3' end of the bIL-12 p40 gene, introduces an BamHI site at the 3' end of the gene. The PCR product was digested with BamHI to yield a fragment 984 base pairs in length corresponding to the bIL-12 p40 gene. The bIL-12 p35 gene is an approximately 665 base pair BglII to BglII fragment synthesized by polymerase chain reaction (PCR) (15,42) using DNA from the plasmid containing IL-12 gene. The upstream primer (5'-GTCAGATCTAATGTGCCCCGCTTCGCAGCCTCCTCCTCATA-3') synthesizes from the 5' end of the bIL-12 p35 gene and introduces an BglII site at the 5' end of the gene. The downstream primer was (5'-CTCAGAGATCTAGGAAGAACTCAGATAGCTCA-3') synthesizes from

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the 3' end of the bIL-12 p35 gene, introduces an BglII site at the 3' end of the gene. The PCR product was digested with BglII to yield a fragment 665 base pairs in length corresponding to the bIL-12 p35 gene.

5

S-SPV-184:

S-SPV-184 is a swinepox virus that expresses five foreign genes. The gene for bovine herpesvirus BHV-1 glycoprotein D (gD) and the gene for E. coli β -galactosidase (lacZ) were inserted into a unique Not I restriction site (Not I linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for bovine cytokine interleukin-12 (bIL-12) p40, p35 and the gene for E. coli β -glucuronidase (uidA) genes were inserted into a unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The gD gene is under the control of the synthetic late/early promoter (LP2EP2), The bIL-12 p40 gene is under the control of the synthetic late promoter (LP2), the bIL-12 p35 gene is under the control of an internal ribosomal entry site (IRES), the uidA gene is under the control of synthetic early promoter (EP2) and the lacZ gene is under the control of the synthetic late promoter (LP1).

S-SPV-184 was derived from S-SPV-148 (Kasza Strain). This was accomplished utilizing the homology vector 870-46 and virus S-SPV-148 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -glucuronidase (x-gluc). The final result of red plaque purification was the recombinant virus designated S-SPV-184. This virus was assayed for β -glucuronidase expression, purity, and

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insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating
5 that the virus is pure, stable, and expressing the foreign genes.

To confirm the expression of the BHV-1 gD and bIL-12 p40, p35 gene products, cells were infected with
10 S-SPV-184 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal goat anti-hIL-12 and polyclonal bovine
15 anti-BHV sera were used to detect expression of bIL-12 and BHV-1 gD specific proteins. The cell lysate from SPV-184 infected cells exhibited bands corresponding to 60 kd, 40kd and 35 kd, which is the expected size of the BHV-1 truncated gD, bIL-12 p40 and p35 proteins.

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S-SPV-184 was assayed for expression of SIV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal goat anti-hIL-12 serum was shown to react specifically with
25 S-SPV-184 plaques and not with S-SPV-148 negative control plaques. All S-SPV-184 observed plaques reacted with serological reagent indicating that the virus was stably expressing the bovine foreign genes. The assays described here were carried out in ESK-4 cells,
30 indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-184 is a recombinant swinepox virus expressing
35 the bovine antigen proteins and is useful as a vaccine in cows against viral infection. S-SPV-184 is also useful for expression of the BHV-1 gD, bIL-12 p40 and

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p35 proteins. A vaccine containing S-SPV-184 stimulates cell mediated immunity and improves growth and weight gain of the animal.

5 **HOMOLOGY VECTOR 870-46. HOMOLOGY VECTOR 870-46.** The
homology vector 870-46 was used to insert foreign DNA
into SPV. It incorporates an E. coli β -glucuronidase
(uidA) marker gene and the bovine cytokine
interleukin-12 (bIL-12) p40 and p35 genes flanked by
10 SPV DNA. When this homology vector was used according
to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
GENERATING RECOMBINANT SPV a virus containing DNA
coding for the foreign genes results. Note that the
 β -glucuronidase (uidA) marker gene is under the control
15 of a synthetic early pox promoter (EP2) and bovine
cytokine interleukin-12 genes (bIL-12) is under the
control of a synthetic late pox promoter (LP2). The
homology vector was constructed utilizing standard
recombinant DNA techniques (22 and 30), by joining
20 restriction fragments from the following sources with
the appropriate synthetic DNA sequences. Plasmid 870-46
was constructed using plasmid 847-42.2C. This plasmid
was previously constructed by inserting the uidA gene
into the unique EcoRI site within the SPV HindIII K
25 genomic fragment. The uidA gene is under the control of
the synthetic early promoter (EP2). The bIL-12 genes
were then inserted into a unique NotI site located
upstream of the uidA gene resulting in plasmid 870-46.
The transcriptional and translational orientation of
30 the bIL-12 genes are the same as the uidA gene.
The bIL-12 p40 gene is an approximately 984 base pair
BamHI to BamHI fragment synthesized by polymerase
chain reaction (PCR) (15,42) using DNA from the plasmid
containing IL-12 gene. The upstream primer
35 (5'-CGTCGGATCCAATGCACCCTCAGCAGTTGGTC -3') synthesizes
from the 5' end of the bIL-12 p40 gene and introduces
an BamHI site at the 5' end of the gene. The downstream

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primer was (5'-GTTGGATCCTAACTGCAGGACACAGATGCCC-3') synthesizes from the 3' end of the bIL-12 p40 gene, introduces an BamHI site at the 3' end of the gene. The PCR product was digested with BamHI to yield a fragment 5 984 base pairs in length corresponding to the bIL-12 p40 gene. The IRES sequence is an 509 base pairs fragment synthesized by PCR using DNA from Novagen®. The upstream primer (5'-AATGGCGCGCCGGTTATTTTCCACCATA-3') synthesized from the 5'-end of the IRES and introduces 10 an AscI site at the 5'-end. The downstream primer was (5'- GTGGGATCCATATTATCATCGTGTTTTTC -3') synthesizes from the 3' end of the IRES, introduces an BamHI site at the 3' end of the gene. The PCR product was digested with AscI and BamHI to yield a fragment 509 base pairs 15 in length corresponding to the IRES sequence. The bIL-12 p35 gene is an approximately 665 base pair BglII to AscI fragment synthesized by polymerase chain reaction (PCR) (15,42) using DNA from the plasmid containing IL-12 gene. The upstream primer (5'- 20 GTCAGATCTAATGTGCCCCGCTTCGCAGCCTCCTCCTCATA -3') synthesizes from the 5' end of the bIL-12 p35 gene and introduces an BglII site at the 5' end of the gene. The downstream primer was (5'- CCTGGCGCGCCTAGGAAGAACTCAGATA -3') synthesizes from the 3' end of the bIL-12 p35 25 gene, introduces an AscI site at the 3' end of the gene. The PCR product was digested with BgIII and AscI to yield a fragment 665 base pairs in length corresponding to the bIL-12 p35 gene.

30 S-SPV-183:

S-SPV-183 is a swinepox virus that expresses two foreign genes. The gene for canine distemper virus (CDV) fusion (F) and the gene for E. coli E. coli β -glucuronidase (uidA) were inserted into a unique Not 35 I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The F gene is

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under the control of the synthetic late promoter (LP1), and the uidA gene is under the control of the synthetic early promoter (EP2).

5 S-SPV-183 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 888-81 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR
10 RECOMBINANT SPV EXPRESSING β -glucuronidase (x-gluc). The final result of blue plaque purification was the recombinant virus designated S-SPV-183. This virus was assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the
15 blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

20 To confirm the expression of the CDV F gene product, cells were infected with S-SPV-183 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis.
25 The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal dog anti-CDV serum was used to detect expression of CDV specific protein. The cell lysate from SPV-183 infected cells exhibited band corresponding to 60kd, which is the expected size of
30 the CDV F protein.

S-SPV-183 is a recombinant swinepox virus expressing the CDV F protein and is useful as a vaccine in dogs against CDV infection. S-SPV-183 is also useful for
35 expression of the CDV F protein.

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HOMOLOGY VECTOR 888-81. The homology vector 888-81 was used to insert foreign DNA into SPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene and the canine distemper virus (CDV) fusion (F) gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β -glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2) and the canine distemper virus fusion (F) gene is under the control of a synthetic late pox promoter (LP1). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 888-81 was constructed using plasmid 847-42.2C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter (EP2). The CDV F gene was then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 888-81. The transcriptional and translational orientation of the CDV F gene is the same as the uidA gene.

The CDV F gene is an approximately 2000 base pair BamHI fragment synthesized by reverse transcription (RT) and polymerase chain reaction (PCR) (15,42) using RNA from the CDV (NVSL challenge strain). The upstream primer (5' - CGGGATCCCATGCACAGGGGAATCCCCAAAAGCTCCACC-3') synthesizes from the 5' end of the CDV F gene and introduces an BamHI site at the 5' end of the gene. The downstream primer was (5' - CGGGATCCTCAGAGTGATCTCACATAGGATTTCTGAAGTTC-3') synthesizes from the 3' end of the CDV F gene, introduces an BamHI site at the 3' end of the gene, and

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was used for reverse transcription and polymerase chain reaction. The PCR product was digested with BamHI to yield a fragment 2000 base pairs in length corresponding to the CDV F gene.

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S-SPV-165:

S-SPV-165 is a swinepox virus that expresses three foreign genes. The genes for bovine cytokine interleukin-12 (IL-12) p35, p40 and the gene for E. coli β -glucuronidase (uidA) were inserted into a unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The p40 gene is under the control of the synthetic late promoter (LP2), the p35 gene is under the control of the synthetic late promoter (LP1) and the uidA gene is under the control of the synthetic early promoter (EP2).

20 S-SPV-165 was derived from S-SPV-001 (Kāsza Strain). This was accomplished utilizing the homology vector 870-56 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -glucuronidase (X-gluc). The final result of blue plaque purification was the recombinant virus designated S-SPV-165. This virus was assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

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To confirm the expression of the bIL-12 gene products, cells were infected with S-SPV-165 and samples of

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infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal goat anti-human IL-12 (hIL-12) serum was used to detect expression of bIL-12 specific proteins. The cell lysate from SPV-165 infected cells exhibited bands corresponding to 40kd, and 35kd, which is the expected size of the bIL-12 p40 and p35 proteins, respectively.

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S-SPV-165 was assayed for expression of IL-12 specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. A polyclonal a polyclonal goat anti-hIL-12 antiserum was shown to react specifically with S-SPV-165 plaques and not with S-SPV-001 negative control plaques. All S-SPV-165 observed plaques reacted with serological reagent indicating that the virus was stably expressing the bIL-12 foreign genes. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

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S-SPV-165 is a recombinant swinepox virus expressing the bIL-12 p40 and p35 proteins and is useful as a vaccine in cows against viral infection. S-SPV-165 is also useful for expression of the bIL-12 proteins. A vaccine containing S-SPV-165 stimulates cell mediated immunity and improves growth and weight gain of the animal.

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HOMOLOGY VECTOR 870-56. The homology vector 870-56 was used to insert foreign DNA into SPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene and the bovine cytokine interleukin-12 (bIL-12) p40 and p35 genes flanked by SPV DNA. When this

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homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β -glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2) and bovine cytokine interleukin-12 genes (bIL-12) is under the control of a synthetic late pox promoters (LP2 and LP1). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 870-56 was constructed using plasmid 847-42.2C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter (EP2). The bIL-12 genes were then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 870-56. The transcriptional and translational orientation of the bIL-12 genes are the same as the uidA gene.

The bIL-12 p40 gene is an approximately 984 base pair BamHI to BamHI fragment synthesized by polymerase chain reaction (PCR) (15,42) using DNA from the plasmid containing IL-12 gene. The upstream primer (5'-CGTCGGATCCAATGCACCCTCAGCAGTTGGTC -3') synthesizes from the 5' end of the bIL-12 p40 gene and introduces an BamHI site at the 5' end of the gene. The downstream primer was (5'-GTTGGATCCTAACTGCAGGACACAGATGCCC-3') synthesizes from the 3' end of the bIL-12 p40 gene, introduces an BamHI site at the 3' end of the gene. The PCR product was digested with BamHI to yield a fragment 984 base pairs in length corresponding to the bIL-12 p40 gene. The bIL-12 p35 gene is an approximately 665 base pair BglII to BglII fragment synthesized by polymerase chain reaction (PCR) (15,42) using DNA from the plasmid containing IL-12 gene. The upstream primer

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(5' - GTCAGATCTAATGTGCCCCGCTTCGCAGCCTCCTCCTCATA - 3') synthesizes from the 5' end of the bIL-12 p35 gene and introduces an BglIII site at the 5' end of the gene. The downstream primer was (5' - CTCAGAGATCTAGGAAGAACTCAGATAGCTCA-3') synthesizes from the 3' end of the bIL-12 p35 gene, introduces an BglIII site at the 3' end of the gene. The PCR product was digested with BglIII to yield a fragment 665 base pairs in length corresponding to the bIL-12 p35 gene.

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S-SPV-164:

S-SPV-164 is a swinepox virus that expresses three foreign genes. The genes for bovine cytokine interleukin-12 (IL-12) p35, p40 and the gene for E. coli β -glucuronidase (uidA) were inserted into a unique Not I site (Not I linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region of the 6.7 kb SPV HindIII K fragment). The p40 gene is under the control of the synthetic late promoter (LP2), the p35 gene is under the control of an internal ribosomal entry site (IRES), and the uidA gene is under the control of the synthetic early promoter (EP2).

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S-SPV-164 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 870-46 and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -glucuronidase (X-gluc). The final result of blue plaque purification was the recombinant virus designated S-SPV-164. This virus was assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of

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purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

5 To confirm the expression of the bIL-12 gene products, cells were infected with S-SPV-164 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN
10 BLOTTING PROCEDURE. A polyclonal goat anti-human IL-12 (hIL-12) serum was used to detect expression of bIL-12 specific proteins. The cell lysate from SPV-164 infected cells exhibited bands corresponding to 40kd, and 35kd, which is the expected size of the bIL-12 p40
15 and p35 proteins, respectively.

S-SPV-164 was assayed for expression of IL-12 specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. A polyclonal a
20 polyclonal goat anti-hIL-12 antiserum was shown to react specifically with S-SPV-164 plaques and not with S-SPV-001 negative control plaques. All S-SPV-164 observed plaques reacted with serological reagent indicating that the virus was stably expressing the
25 bIL-12 foreign genes. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

30 S-SPV-164 is a recombinant swinepox virus expressing the bIL-12 p40 and p35 proteins and is useful as a vaccine in cows against viral infection. S-SPV-164 is also useful for expression of the bIL-12 proteins. A vaccine containing S-SPV-164 stimulates cell mediated
35 immunity and improves growth and weight gain of the animal.

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HOMOLOGY VECTOR 870-46. The homology vector 870-46 was used to insert foreign DNA into SPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene and the bovine cytokine interleukin-12 (bIL-12) p40 and p35 genes flanked by SPV DNA. When this

5 homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β -glucuronidase (uidA) marker

10 gene is under the control of a synthetic early pox promoter (EP2) and bovine cytokine interleukin-12 genes (bIL-12) is under the control of a synthetic late pox promoter (LP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and

15 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 870-46 was constructed using plasmid 847-42.2C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site

20 within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter (EP2). The bIL-12 genes were then inserted into a unique NotI site located upstream of the uidA gene resulting in plasmid 870-46. The transcriptional

25 and translational orientation of the bIL-12 genes are the same as the uidA gene.

The bIL-12 p40 gene is an approximately 984 base pair BamHI to BamHI fragment synthesized by polymerase chain reaction (PCR) (15,42) using DNA from the plasmid

30 containing IL-12 gene. The upstream primer (5'-CGTCGGATCCAATGCACCCTCAGCAGTTGGTC -3') synthesizes from the 5' end of the bIL-12 p40 gene and introduces an BamHI site at the 5' end of the gene. The downstream primer was (5'-GTTGGATCCTAACTGCAGGACACAGATGCCC-3')

35 synthesizes from the 3' end of the bIL-12 p40 gene, introduces an BamHI site at the 3' end of the gene. The PCR product was digested with BamHI to yield a fragment

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984 base pairs in length corresponding to the bIL-12 p40 gene. The IRES sequence is an 509 base pairs fragment synthesized by PCR using DNA from Novagen[®]. The upstream primer (5'-AATGGCGCGCCGGTTATTTTCCACCATA-3') synthesized from the 5'-end of the IRES and introduces an AscI site at the 5'-end. The downstream primer was (5'- GTGGGATCCATATTATCATCGTGTTTTC -3') synthesizes from the 3' end of the IRES, introduces an BamHI site at the 3' end of the gene. The PCR product was digested with AscI and BamHI to yield a fragment 509 base pairs in length corresponding to the IRES sequence. The bIL-12 p35 gene is an approximately 665 base pair BglII to AscI fragment synthesized by polymerase chain reaction (PCR) (15,42) using DNA from the plasmid containing IL-12 gene. The upstream primer (5'- GTCAGATCTAATGTGCCCGCTTCGCAGCCTCCTCCTCATA -3') synthesizes from the 5' end of the bIL-12 p35 gene and introduces an BglII site at the 5' end of the gene. The downstream primer was (5'- CCTGGCGCGCCTAGGAAGAACTCAGATA -3') synthesizes from the 3' end of the bIL-12 p35 gene, introduces an AscI site at the 3' end of the gene. The PCR product was digested with BglII and AscI to yield a fragment 665 base pairs in length corresponding to the bIL-12 p35 gene.

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S-SPV-176 :

S-SPV-176 is a swinepox virus that expresses four foreign genes. The gene for bovine respiratory syncytial virus glycoprotein (G) and the gene for E. coli β -galactosidase (lacZ) were inserted into a unique Not I restriction site (NotI linkers inserted into a unique AccI restriction site in the O1L ORF of the SPV HindIII M fragment). The gene for bovine respiratory syncytial virus fusion (F) and the gene for E. coli β -glucuronidase (uidA) were inserted into a unique NotI site (NotI linkers inserted into a unique EcoRI restriction site within an approximately 3.2 kb region

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of the 6.7 kb SPV HindIII K fragment). . The gene for bovine respiratory syncytial virus glycoprotein G is under the control of the synthetic late/early promoter (LP2EP2), . The gene for bovine respiratory syncytial virus fusion (F) is under the control of the synthetic late promoter (LP1). The lacZ gene is under the control of the synthetic late promoter (LP1), the uidA gene is under the control of the synthetic early promoter (EP2).

10

S-SPV-176 was derived from S-SPV-020 (Kasza Strain). This was accomplished utilizing the homology vector 888-38.9 and virus S-SPV-020 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV.

15

The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -glucuronidase (SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of blue plaque purification was the recombinant virus designated S-SPV-176 . This

20

virus was assayed for β -galactosidase and β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign genes.

25

To confirm the expression of bovine respiratory syncytial virus glycoprotein (G) and fusion (F) gene products, cells were infected with S-SPV-176 and samples of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Bovine anti-FITC (Accurate Chemicals) or a F specific Mab (Mab19) was used to detect expression of BRSV specific proteins. The cell lysate from SPV-176 infected cells exhibited bands corresponding to 80 kd

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to 90 kd and 70 kd which is the expected size of the bovine respiratory syncytial virus glycoprotein (G) and fusion (F) gene products. The assay described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

S-SPV-176 is a recombinant swinepox virus expressing the PRRS ORF5 and ORF6 proteins and is useful as a vaccine in swine against PRRS infection. S-SPV-134 is also useful for expression of the PRRS ORF5 and ORF6 proteins.

HOMOLOGY VECTOR 888-38.9. The homology vector 888-38.9 was used to insert foreign DNA into SPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene and the bovine respiratory syncytial virus fusion (F) gene flanked by SPV DNA. When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the β -glucuronidase (uidA) marker gene is under the control of a synthetic early pox promoter (EP2) and the bovine respiratory syncytial virus fusion (F) gene is under the control of a synthetic late pox promoter (LP1). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 888-38.9 was constructed using plasmid 847-42.7C. This plasmid was previously constructed by inserting the uidA gene into the unique EcoRI site within the SPV HindIII K genomic fragment. The uidA gene is under the control of the synthetic early promoter (EP2). The bovine respiratory syncytial virus fusion (F) gene was then inserted into a unique

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d o w n s t r e a m p r i m e r
(5'-CTCTGGATCCTACAGCCATGAGGATGATCATCAGC-3') synthesizes
from the 3' end of the fusion (F) gene, introduces an
BamHI site at the 3' end of the gene, and was used for
5 reverse transcription and polymerase chain reaction.
The PCR product was digested with Bam HI to yield a
fragment 1723 base pairs in length corresponding to the
fusion (F) gene.

10 S-SPV-143:

S-SPV-143 is a swinepox virus that expresses three
foreign genes. The genes for bovine viral diarrhea
virus type 1 (BVDV1) E2 glycoprotein and the gene for
15 E. coli β -galactosidase (lacZ) were inserted into a
unique Not I restriction site (Not I linkers inserted
into a unique AccI restriction site in the OIL ORF of
the SPV HindIII M fragment). The genes for bovine
viral diarrhea d virus type 1(BVDV1) E glycoprotein,
20 bovine viral diarrhea virus type 2(BVDV2) E2
gylcoprotein and the E. coli β -glucuronidase (uidA)
marker gene were inserted into unique Bam HI, Not I and
Pst I site respectively (sites originating from a
synthetic polylinker inserted into a unique EcoRI
25 restriction site within an approximately 3.2 kb region
(SEQ ID NO) of the 6.7 kb SPV HindIII K Fragment). The
E gene and the lacZ gene are under the control of the
synthetic late promoter (LP1), and the uidA gene is
under the control of the synthetic early promoter.

30

S-SPV-143 was derived from S-SPV-051 (Kasza Strain).
This was accomplishing utilizing the homology vector
874-06.20B and virus S-SPV-051 in the HOMOLOGOUS
RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV.
35 The BVDV type 1 E2 gene and the lacZ gene in the
HindIII M site were already present in virus S-SPV-051.
The transfection stock was screened by the SCREEN FOR

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RECOMBINANT SPV EXPRESSING β -GLUCURONIDASE (x-Gluc
ASSAY and SCREEN FROM RECOMBINANT HERPESVIRUS
EXPRESSING ENZYMATIC MARKER GENES). The final result
of green plaque purification was the recombinant virus
5 designated S-SPV-143. The virus was assayed for β -
glucuronidase expression, purity, and insert stability
by multiple passages monitored by the green plaque
assay as describes in Materials and Methods. After the
initial four rounds of purification, all plaques
10 observed are green indicating that the virus is pure
stable, and expressing the foreign genes.

To confirm the expression of the BVDV type 2 E2 and
type 1 E gene products, cells were infected with S-SPV-
15 143 and samples of infected cell lysates were subjected
to SDS polyacrylamide gel electrophoresis. The gel was
blotted and analyzed using the WESTERN BLOTTING
PROCEDURE. A monoclonal mouse anti-E2 (type 2) of a
monoclonal mouse anti-E (type 1) serum was used to
20 detect expression of BVDV-specific proteins. The cell
lysate from S-SPV-143 infected cell exhibited bands
corresponding to 53 kd and 42 kd, which is the
expected size of the E2 glycoprotein (53 kd) but
slightly smaller than the expected size of the E
25 glycoprotein (48 kd).

S-SPV-143 was assayed for expression of BVDV-specific
antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE
EXPRESSING IN RECOMBINANT SPV. Monoclonal mouse anti-E2
30 sera were shown to react specifically with S-SPV-143
plaques and not with S-SPV-001 negative control
plaques. All S-SPV-143 observed plaques reacted with
both a type 1 E2 and a type 2 E2 serum indicating that
the virus was stably expressing the BVDV E2 foreign
35 genes. No reagent is currently available that reacts
specifically with the E glycoprotein in a black plaque
assay. The assays described here were carried out in

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ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production for SPV recombinant vaccines.

- 5 S-S.V.-143 is a recombinant swinepox virus expressing the types 1 & 1 BVDV E2 glycoproteins and the type 1 E glycoprotein and is useful as a vaccine against BVDV infection.
- 10 HOMOLOGY VECTOR 874-06.20B. The homology vector 874-06.20B was used to insert foreign DNA into SPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene and the bovine viral diarrhea virus (BVDV) type 1 E gene and the BVDV type 2 E2 gen flanked by SPV DNA.
- 15 When this homology vector was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note the β -glucuronidase marker gene is under the control of a synthetic early
- 20 pox promoter (EP2), the E gene is under control of the late synthetic pox promoter (LP1) and the BVDV E2 gene is under the control of a synthetic late/early pox promoter (LP2EP2). The homology vector was constructed utilizing standard recombinant DNA techniques (22 and
- 25 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. Plasmid 874-06.20B was constructed using plasmid 847-90.2B. This plasmid was previously constructed by inserting the BVDV type 2 E2 gene under
- 30 the control of the synthetic late/early promoter (LP2EP2) into a unique Not I site flanked by the EP2-uidA cassette downstream of the Not I site. The Not I originated from a synthetic polylinker inserted into the unique Eco RI site of the SPV HindIII K genomic
- 35 fragment. The EP 2-uidA cassette had previously been inserted into the blunt-ended resulting in plasmid 874-

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06.20B. The translational orientation of the E gene is the same as the E 2 and uidA genes.

The BVDV E gene is an approximately 744 base pair (63
5 bp signal sequence +681 bp coding sequence) Eco RI to
Bam HI fragment synthesized by reverse transcription
(RT) and polymerase chain reaction using RNA from the
BVDV1-Singer strain. The upstream primer (5'-
CCATGAATTCGCTGGAAAAAGCATTGCTGGCATGGGC-3'; 8/96.3)
10 synthesizes from the 5' end of the BVDV E gene signal
sequence and introduces an Eco RI site at the 5' end of
the gene. The downstream primer (5'-
TTCGGATCCTTACGCGTATGCTCCAAACCACGT-3'; 8/96.4)
synthesizes from the 3' end of the E gene and
15 introduces a Bam HI site at the 3' end of the gene. The
PCR product was digested with Eco RI and Bam HI to
yield a fragment 744 base pairs in length
corresponding to the BVDV1 E gene.

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REFERENCES:

1. C. Bertholet, et al., *EMBO Journal* 5, 1951-1957 (1986).
2. R. A. Bhat, et al., *Nucleic Acids Research* 17, 1159-1176 (1989).
3. D. A. Boyden, et al., *Infection and Immunity* 57, 3808-3815 (1989).
4. D. B. Boyle and B. E. H. Coupar, *Virus Research* 10, 343-356 (1988).
5. R. M. Buller, et al., *Nature* 317, 813-815 (1985).
6. K. J. Cremer, et al., *Science* 228, 737-739 (1985).
7. A. J. Davidson and B. Moss, *J. Mol. Biol.* 210, 749-769 (1989).
8. A. J. Davidson and B. Moss, *J. Mol. Biol.* 210, 771-784 (1989).
9. P. L. Earl, et al., *Journal of Virology* 64, 2448-2451 (1990).
10. J. J. Esposito, et al., *Virology* 165, 313 (1988).
11. F. A. Ferrari, et al., *J. of Bacteriology* 161, 556-562 (1985).
12. C. Flexner, et al., *Vaccine* 8, 17-21 (1990).
13. S. J. Goebel, et al., *Virology* 179, 247-266 (1990).
14. U. Gubler and B. J. Hoffman, *Gene* 25, 263-269 (1983).
15. M. A. Innis, et al., *PCR Protocols A Guide to Methods and Applications*, 84-91, Academic Press, Inc., San Diego (1990).
16. S. Joshi, et al., *Journal of Virology*. 65, 5524-5530 (1991).

WO 98/04684

PCT/US97/12212

-334-

17. L. Kasza, et al., *Am. J. Vet. Res.* 21, 269-273 (1960).
18. L. Kasza, *Diseases of Swine*, 254-260, Ed. A.D. Leman, et al., The Iowa State University Press, Ames, Iowa (1981).
19. B. G. Klupp and T. C. Mettenleiter, *Virology* 182, 732-741 (1991).
20. U. K. Laemli, *Nature* 227, 680-685 (1970).
21. B. Lominiczi, et al., *Journal of Virology* 49, 970-979 (1984).
22. T. Maniatis, et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1982).
23. R. F. Massung, and R. W. Moyer, *Virology* 180, 347-354 (1991).
24. R. F. Massung, and R. W. Moyer, *Virology* 180, 355-364 (1991).
25. B. Moss, *Science* 252, 1662-1667 (1991).
26. E. A. Petrovskis, et al., *Journal of Virology* 59, 216-223 (1986).
27. A. K. Robbins et al., *Journal of Virology* 58, 339-347 (1986).
28. A. K. Robbins et al., *Journal of Virology* 61, 2691-2701 (1987).
29. A. C. R. Samson, *Journal of Virology* 67, 1199-1203 (1986).
30. J. Sambrook, et al., *Molecular Cloning A Laboratory Manual Second Edition*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989).
31. Sheffy, et al., *Proceedings 65th Annual Meeting of the United States*

WO 98/04684

PCT/US97/12212

-335-

- Livestock Association 65, 347-353 (1961).
32. W. M. Schnitzlein and D. N. Tripathy, *Virology* 181, 727-732, (1991).
33. J. Taylor, et al., *Vaccine* 9, 190-193, (1991).
34. M. Wachsman, et al., *Journal of General Virology* 70, 2513-2520 (1989).
35. M. W. Wathen, et al., *Journal of Virology* 51, 57-62 (1984).
36. M. Weerasinge, *Journal of Virology* 65, 5531-5534 (1991).
37. T. Ben-Porat, et al., *Journal of Virology*, volume 154, 325-334 (1986).
38. F. Zuckerman, et al., *Vaccination and Control of Adjesky's Disease*, J. T. Van Oirchot (ed.). Kluwer Academic Publishers, London, pp. 107-117 (1989).
39. Paolette, et al., *Journal of Virology*, volume 66, pp. 3424-3434 (June, 1992).
40. M. W. Mellencamp, et al., *Journal of Clinical Microbiology*, volume 27, pp. 2208-2213 (1989).
41. L. A. Herzenberg, et al., *Selected Methods in Cellular Immunology*, Freeman Publ. Co., San Francisco, 351-372 (1980).
42. Katz et al., *Journal of Virology* 64, 1808-1811 (1990).
43. Taniguchi, T., et al., *Biochem. Biophys. Res. Commun.* 115 1040-1047 (1983).
44. Cochran, M.D. and Macdonald, R.D., WO 93/02104, published February 4, 1993.

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PCT/US97/12212

-336-

45. Galibert, F., et al., *Nature* 281, 646-650 (1979).
46. Thomsen, D.R., et al., *Proc. Natl. Acad. Sci. USA* 81, 659-663 (1984).
47. Catalog Number 267402, Beckman Instruments, Inc., Fullerton California.
48. Whalley, J.M., et al., *Journal of General Virology* 57 307-323 (1981).
49. Collett, M.S., et al., *Virology* 165 200-208 (1988).
50. Schodel, F. et al., *Journal of Virology* 66, 106-114 (1992).
51. Cochran, M.D., WO 93/25665, published December 23, 1993.
52. C.A. Hjerpe, *The bovine Respiratory Disease Complex*. Ed. by J.L. Howard, Philadelphia, W.B. Saunders Co., 670-680 (1986).
53. F. Fenner, et al., *Veterinary Virology*. Academic Press, Inc., Orlando Florida, 183-202 (1987).
54. A. Leutz, et al., *EMBO Journal* 8: 175-182 (1989).
55. M.J. Sekellick, et al., *Journal of Interferon Research* 14: 71-79 (1994).
56. S.J. Child, et al., *Virology* 174: 625-629 (1990).
57. G.P. Johnson, et al. *Virology* 196: 381-401 (1993).
58. R.F. Massung, et al. *Virology* 201: 215-240 (1994).
60. Child, S.J. et al., *Virology* 174, 625-629 (1990).

WO 98/04684

PCT/US97/12212

-337-

61. T.R. Phillips, et al., J. Virology
64, 4605-4613 (1990).
62. Digby, M.R., and Lowenthal, J.W.,
Journal of Interferon and Cytokine
Research 15: 939 (1996).
63. G.J. Winston et al., Proc. Natl.
Acad. Sci. USA 90: 1272 (1993).

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANTS: Cochran Ph.D., Mark D
Junker M.S., David E
- (ii) TITLE OF INVENTION: Recombinant Swinepox Virus
- (iii) NUMBER OF SEQUENCES: 101
- (iv) CORRESPONDENCE ADDRESS:
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- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
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 - (C) TELEX: 422523

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3164 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Swinepox virus
 - (B) STRAIN: Kasza Strain
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 1..231
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 339..1628

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(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1683..3161

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AAG CTT CTC AAT TAT GAT AAT TTT TTA AGA TTA AAA AAT TTA GTA ATG	48
Lys Leu Leu Asn Tyr Asp Asn Phe Leu Arg Leu Lys Asn Leu Val Met	
1 5 10 15	
TAT GGA TCA CAT ATA GAA AAT ATT ATC AAA AAT ACA TAT ATG TAT TAT	96
Tyr Gly Ser His Ile Glu Asn Ile Ile Lys Asn Thr Tyr Met Tyr Tyr	
20 25 30	
TCT AAC ATT GAT AAA GCG ATT TAT GTA ATT ATG AAG CAC TGC AAG AAA	144
Ser Asn Ile Asp Lys Ala Ile Tyr Val Ile Met Lys His Cys Lys Lys	
35 40 45	
CAT AGT TAC TGG ATG AGG ATT CCT ATA GAA ATA CAA CGA TAT ATA TTA	
192	
His Ser Tyr Trp Met Arg Ile Pro Ile Glu Ile Gln Arg Tyr Ile Leu	
50 55 60	
TTA CAT TTA ACA ATG AAG GAC TTA TCA ATA ATA CTT AAG TAATAATGTC	241
Leu His Leu Thr Met Lys Asp Leu Ser Ile Ile Leu Lys	
65 70 75	
ATAATATTGA AAAAAAATTT TTTTCTAGT AATGTGGCTA TTATTAGTAG CCCATGAATA	301
CATTTTGGTT ATCGTTTAAA TAGTTTGTA GAAGGAA ATG GAT AAT ATA AGA AGA	356
Met Asp Asn Ile Arg Arg	
1 5	
ATA ATA TCA AAT ATA AAA CAG GAT GAT AAT ATA GCC ACT GAT ATG TTA	404
Ile Ile Ser Asn Ile Lys Gln Asp Asp Asn Ile Ala Thr Asp Met Leu	
10 15 20	
GCT ACA TTT TTA AGT TCA TCG TTG CAC GTA TTT AAA TTA AAA GAG TTG	452
Ala Thr Phe Leu Ser Ser Ser Leu His Val Phe Lys Leu Lys Glu Leu	
25 30 35	
AAA GAA ATT GTA TTA TTA CTG CTT AAT AAA GGT GCT AAT TTA AAT GGG	500
Lys Glu Ile Val Leu Leu Leu Leu Asn Lys Gly Ala Asn Leu Asn Gly	
40 45 50	
ATA TCT ATA TAT GAT AAA ACA CCA TTT CAT TGT TAT TTT ACA TTT AAT	548
Ile Ser Ile Tyr Asp Lys Thr Pro Phe His Cys Tyr Phe Thr Phe Asn	
55 60 65 70	
ACG AAT GTT ACA ATT AAA GTA ATA AAG TTT CTT ATT TAT CAT GGT GGT	596
Thr Asn Val Thr Ile Lys Val Ile Lys Phe Leu Ile Tyr His Gly Gly	
75 80 85	
GAC ATT AAC AGT GTA CAT AGA TGT GGA GAC ACC ATA TTG CAT AAA TAC	644
Asp Ile Asn Ser Val His Arg Cys Gly Asp Thr Ile Leu His Lys Tyr	
90 95 100	
CTT GGT AAT GAG AAT ATA GAT TAT AAA GTT GTT GAG TTT TTA ATA AGA	692
Leu Gly Asn Glu Asn Ile Asp Tyr Lys Val Val Glu Phe Leu Ile Arg	
105 110 115	
AAA GGA TTT GAT GTA TGT AAA CTA AAT AAT AGT CTG AAG AAT CCT ATT	740
Lys Gly Phe Asp Val Cys Lys Leu Asn Asn Ser Leu Lys Asn Pro Ile	
120 125 130	

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CAT ATA TTT ACA ATT AGA CAC ATC AAT AAC ACT AAT TTA AAT ATA TTG His Ile Phe Thr Ile Arg His Ile Asn Asn Thr Asn Leu Asn Ile Leu 135 140 145 150	788
AAT TTG CTT TGT TCG CAT ATA AAA CAT GAA TAT AAT AAA AAT GAT GAA Asn Leu Leu Cys Ser His Ile Lys His Glu Tyr Asn Lys Asn Asp Glu 155 160 165	836
ATG ATG TCG ATA TTA AAC ACG ATG TTA AAC TAT TGT CAC GAC GAT TAT Met Met Ser Ile Leu Asn Thr Met Leu Asn Tyr Cys His Asp Asp Tyr 170 175 180	884
ACA TGT TTT TCG GCG GTC CCA TAT ACT ATA GAT ATC ACA ACC ATA AAC Thr Cys Phe Ser Ala Val Pro Tyr Thr Ile Asp Ile Thr Thr Ile Asn 185 190 195	932
TAT AGA GAT AAA TTA GGA TAT TCT CCT GTT GTG TAT GCA TCT ACC ACG Tyr Arg Asp Lys Leu Gly Tyr Ser Pro Val Val Tyr Ala Ser Thr Thr 200 205 210	980
GAT AAA ACT ATC TTG GTG GAT TAT CTT ATT AAA TTA GGA GCA AAC ATG Asp Lys Thr Ile Leu Val Asp Tyr Leu Ile Lys Leu Gly Ala Asn Met 215 220 225 230	1028
AAC ATA ACA ACG AAC GAT GGT AAT ACA TGT GGT TCG TTT GCT GTA ATG Asn Ile Thr Thr Asn Asp Gly Asn Thr Cys Gly Ser Phe Ala Val Met 235 240 245	1076
AAT TGT AAC AGG GAT ATT AAT AGA CTA TTT CTT AAT CAA AAT CCA AAT Asn Cys Asn Arg Asp Ile Asn Arg Leu Phe Leu Asn Gln Asn Pro Asn 250 255 260	1124
ATA GAA ACT ATA TAT AAT ACA TTG AAG ATA TTA TCG GAG AAT ATA GTA Ile Glu Thr Ile Tyr Asn Thr Leu Lys Ile Leu Ser Glu Asn Ile Val 265 270 275	1172
TTC ATA GAC GGA TGT GAT GTA CGT ACG AAT ATG GTT AAA AAA ATA CTA Phe Ile Asp Gly Cys Asp Val Arg Thr Asn Met Val Lys Lys Ile Leu 280 285 290	1220
ATG TAC GGA TTT ACT TTA GAT CCA CTA TTT TAC AAG AAC CAC GAT ATC Met Tyr Gly Phe Thr Leu Asp Pro Leu Phe Tyr Lys Asn His Asp Ile 295 300 305 310	1268
ATT GTT GAA TAT TTT TCA AGT AGT ATT AAA AAG TAT AAT AAG ATT ATT Ile Val Glu Tyr Phe Ser Ser Ser Ile Lys Lys Tyr Asn Lys Ile Ile 315 320 325	1316
TTA CAA ATG ATC GAT GAG AAA ATT GGG AAT AGA TCC GTA TAC GAT ATT Leu Gln Met Ile Asp Glu Lys Ile Gly Asn Arg Ser Val Tyr Asp Ile 330 335 340	1364
ATA TTT ACT AAA TCA AAT ACA GGT ATG GAT GTT AGA TAT GTA TGT AAT Ile Phe Thr Lys Ser Asn Thr Gly Met Asp Val Arg Tyr Val Cys Asn 345 350 355	1412
GAT ATC ATT ATA AAA TAT GCA AGT GTT AAA TAT TAT GGA TCT TTA ATA Asp Ile Ile Ile Lys Tyr Ala Ser Val Lys Tyr Tyr Gly Ser Leu Ile 360 365 370	1460
AAA CGT TTG ATA TAT CAT TCT AAG AAA AGG AAG CGA AAT ATA TTA AAA Lys Arg Leu Ile Tyr His Ser Lys Lys Arg Lys Arg Asn Ile Leu Lys 375 380 385 390	1508
GCT ATA CAT GCG ATG GAG AAT AAC ACA ACC TTG TGG AAT TAC CTA CCA Ala Ile His Ala Met Glu Asn Asn Thr Thr Leu Trp Asn Tyr Leu Pro 395 400 405	1556

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TTG GAA GTA AAA ATG TAT ATT ATG GAT TTC TTA CCC GAT ACT GAT ATA Leu Glu Val Lys Met Tyr Ile Met Asp Phe Leu Pro Asp Thr Asp Ile 410 415 420	1604
ACT AAC ATT CTT TTT ATG AAA AAA TGAAAATATA TACATAAGAC AGGGAATTCC Thr Asn Ile Leu Phe Met Lys Lys 425 430	1658
TATTGTTTTT TTATATAGGG GAAA ATG GAT AAT CTA TAC CGA TAT ATT ACT Met Asp Asn Leu Tyr Arg Tyr Ile Thr 1 5	1709
GTA TCC GAT ACA GTG GAC GTA GAT AAT GTA AGA AAA TTA TTA TCT TCG Val Ser Asp Thr Val Asp Val Asp Asn Val Arg Lys Leu Leu Ser Ser 10 15 20 25	1757
TGT AAT ATC GAC GTA GTC TCT ACA ATA TTT CAA AAA TAT CTT CAT AGA Cys Asn Ile Asp Val Val Ser Thr Ile Phe Gln Lys Tyr Leu His Arg 30 35 40	1805
AAC GAT ATT AAA TTA GAT ATC GTT GAA GAG TTT GTG AAT AAC GGA GCT Asn Asp Ile Lys Leu Asp Ile Val Glu Glu Phe Val Asn Asn Gly Ala 45 50 55	1853
AAA CTG AAT GGG AAA GAT TTT AAC GAT AAA AAT ATA CCA TTG TGT ACA Lys Leu Asn Gly Lys Asp Phe Asn Asp Lys Asn Ile Pro Leu Cys Thr 60 65 70	1901
TTA TTA TCT AAT AAA TTC ATA GAT TAT AAT AGT GCC ATC GAT ATA ACA Leu Leu Ser Asn Lys Phe Ile Asp Tyr Asn Ser Ala Ile Asp Ile Thr 75 80 85	1949
AGT TTT ATG ATT ACA CAT GGA GCG GAT ATA AAT AAG AGA AAT AAG GAT Ser Phe Met Ile Thr His Gly Ala Asp Ile Asn Lys Arg Asn Lys Asp 90 95 100 105	1997
GGG CGT ACT CCT ATA TTT TGT TTA CTA CAT AAT TCT ACA TTA AAT AAT Gly Arg Thr Pro Ile Phe Cys Leu Leu His Asn Ser Thr Leu Asn Asn 110 115 120	2045
TTA GAA TTT GTA TCT TTT ATG ATA GAC CAT GGT GCA GAT ATT ACA ATA Leu Glu Phe Val Ser Phe Met Ile Asp His Gly Ala Asp Ile Thr Ile 125 130 135	2093
GTT GAT GGA TTC GGG TTC ACA TCA TTA CAA ATA TAT TTA CAA TCA TCA Val Asp Gly Phe Gly Phe Thr Ser Leu Gln Ile Tyr Leu Gln Ser Ser 140 145 150	2141
AAT GTA CAA TTA GAT TTG GTT GAG TTA TTG ATA CAA AAG GGG GTC GAT Asn Val Gln Leu Asp Leu Val Glu Leu Leu Ile Gln Lys Gly Val Asp 155 160 165	2189
GTA AAT ATA CAT AAT AAT TGG TTC TAT TAC AAT ACA TTA CAT TGT TAT Val Asn Ile His Asn Asn Trp Phe Tyr Tyr Asn Thr Leu His Cys Tyr 170 175 180 185	2237
ATA AAG AAA AAT TAT AAC CGT ATT AAT ATG GAT ATT ATA AAA TAT ATA Ile Lys Lys Asn Tyr Asn Arg Ile Asn Met Asp Ile Ile Lys Tyr Ile 190 195 200	2285
ATG GAC AAT GGA TTT ACA ATT AAT GAG AAT AAA TTT ACC AAA TCA ACA Met Asp Asn Gly Phe Thr Ile Asn Glu Asn Lys Phe Thr Lys Ser Thr 205 210 215	2333
TTT TTA GAT ATA TTG GTA TCA ATT ATT GAT AGT AAA AAC TTT GAC TCA Phe Leu Asp Ile Leu Val Ser Ile Ile Asp Ser Lys Asn Phe Asp Ser 220 225 230	2381

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AAC GTT GTT GAT TTT ATA TTA AAA TAT ATT GAT ATT AAT GAA AAG AAT Asn Val Val Asp Phe Ile Leu Lys Tyr Ile Asp Ile Asn Glu Lys Asn 235 240 245	2429
ATT TTT GAT TTT ACG CCA TTA TAC TGT TCT GTA GAT GCA AAT AAT GAA Ile Phe Asp Phe Thr Pro Leu Tyr Cys Ser Val Asp Ala Asn Asn Glu 250 255 260 265	2477
AAG ATG TGT TCT TAT TTA CTA AAA AAG AAT GCA GAC CCT AAT ATT ATC Lys Met Cys Ser Tyr Leu Leu Lys Lys Asn Ala Asp Pro Asn Ile Ile 270 275 280	2525
ACA GTA TTT GGT GAA ACG TGT ATA CTA ACA GCT ATC AAT AAT CAT AAT Thr Val Phe Gly Glu Thr Cys Ile Leu Thr Ala Ile Asn Asn His Asn 285 290 295	2573
AAA AAT ATA TTA TAT AAA CTA TTA AAT TAT GAT ATA GAT ATA AAT ACT Lys Asn Ile Leu Tyr Lys Leu Leu Asn Tyr Asp Ile Asp Ile Asn Thr 300 305 310	2621
ATC CAA AAT ACA TTA TTT AAA CTG GAA CAA GAT ATT ATT AAC TCT ACC Ile Gln Asn Thr Leu Phe Lys Leu Glu Gln Asp Ile Ile Asn Ser Thr 315 320 325	2669
ATA GAT ACT TAC TAT TAC AAT AAT CTT GTT AAA AAA GAA CAT TTT ATA Ile Asp Thr Tyr Tyr Tyr Asn Asn Leu Val Lys Lys Glu His Phe Ile 330 335 340 345	2717
AAA TTA TTT CTA GCC TAC ATA GTT AAG AGG TAT GAA AAA AAT ATA GGA Lys Leu Phe Leu Ala Tyr Ile Val Lys Arg Tyr Glu Lys Asn Ile Gly 350 355 360	2765
ATA TTA TTT CTT GAT TAT CCC ACT CTT GGT GAA TAT TTC GTG AAA TTT Ile Leu Phe Leu Asp Tyr Pro Thr Leu Gly Glu Tyr Phe Val Lys Phe 365 370 375	2813
ATA GAT ACG TGT ATG ATG GAA ATA TTT GAG ATG AAA TCA GAT AAG GCT Ile Asp Thr Cys Met Met Glu Ile Phe Glu Met Lys Ser Asp Lys Ala 380 385 390	2861
GGT AAT ACG GAT ATA TAT TCT ATT ATA TTT ACG AAT AAG TAT ATT CCT Gly Asn Thr Asp Ile Tyr Ser Ile Ile Phe Thr Asn Lys Tyr Ile Pro 395 400 405	2909
ATC CCA TAT ATA ACG TGT AAA AAG CTA AAG AAA TAC GAA TCC TTT GTT Ile Pro Tyr Ile Thr Cys Lys Lys Leu Lys Lys Tyr Glu Ser Phe Val 410 415 420 425	2957
GTA TAT GGA ACC GAA ATA AAA TCA ATA ATA AAA TCT TCA AAG ATT AGA Val Tyr Gly Thr Glu Ile Lys Ser Ile Ile Lys Ser Ser Lys Ile Arg 430 435 440	3005
TAT GCG AGT GTT ATA AAA GTA ACG GAG TAT ATC ACA TCT ATC TGT TCG Tyr Ala Ser Val Ile Lys Val Thr Glu Tyr Ile Thr Ser Ile Cys Ser 445 450 455	3053
GAA GAA ACT AGT TTA TGG AAC AGC ATC CCA ATT GAG ATA AAA CAT AAG Glu Glu Thr Ser Leu Trp Asn Ser Ile Pro Ile Glu Ile Lys His Lys 460 465 470	3101
ATT ATT AAT AAT ATA AAC AAT CAT GAT ATG TAT ATA TTA TAT AAA AAT Ile Ile Asn Asn Ile Asn Asn His Asp Met Tyr Ile Leu Tyr Lys Asn 475 480 485	3149
AGA AAA AAA AAA TAA Arg Lys Lys Lys 490	3164

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 77 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Lys Leu Leu Asn Tyr Asp Asn Phe Leu Arg Leu Lys Asn Leu Val Met
 1              5              10              15
Tyr Gly Ser His Ile Glu Asn Ile Ile Lys Asn Thr Tyr Met Tyr Tyr
                20              25              30
Ser Asn Ile Asp Lys Ala Ile Tyr Val Ile Met Lys His Cys Lys Lys
                35              40              45
His Ser Tyr Trp Met Arg Ile Pro Ile Glu Ile Gln Arg Tyr Ile Leu
 50              55              60
Leu His Leu Thr Met Lys Asp Leu Ser Ile Ile Leu Lys
 65              70              75

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 430 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

Met Asp Asn Ile Arg Arg Ile Ile Ser Asn Ile Lys Gln Asp Asp Asn
 1              5              10              15
Ile Ala Thr Asp Met Leu Ala Thr Phe Leu Ser Ser Ser Leu His Val
                20              25              30
Phe Lys Leu Lys Glu Leu Lys Glu Ile Val Leu Leu Leu Leu Asn Lys
                35              40              45
Gly Ala Asn Leu Asn Gly Ile Ser Ile Tyr Asp Lys Thr Pro Phe His
 50              55              60
Cys Tyr Phe Thr Phe Asn Thr Asn Val Thr Ile Lys Val Ile Lys Phe
 65              70              75              80
Leu Ile Tyr His Gly Gly Asp Ile Asn Ser Val His Arg Cys Gly Asp
                85              90              95
Thr Ile Leu His Lys Tyr Leu Gly Asn Glu Asn Ile Asp Tyr Lys Val
                100              105              110
Val Glu Phe Leu Ile Arg Lys Gly Phe Asp Val Cys Lys Leu Asn Asn
                115              120              125
Ser Leu Lys Asn Pro Ile His Ile Phe Thr Ile Arg His Ile Asn Asn
                130              135              140
Thr Asn Leu Asn Ile Leu Asn Leu Leu Cys Ser His Ile Lys His Glu

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145		150		155		160
Tyr Asn Lys Asn Asp Glu Met Met Ser Ile Leu Asn Thr Met Leu Asn						
		165		170		175
Tyr Cys His Asp Asp Tyr Thr Cys Phe Ser Ala Val Pro Tyr Thr Ile						
		180		185		190
Asp Ile Thr Thr Ile Asn Tyr Arg Asp Lys Leu Gly Tyr Ser Pro Val						
		195		200		205
Val Tyr Ala Ser Thr Thr Asp Lys Thr Ile Leu Val Asp Tyr Leu Ile						
		210		215		220
Lys Leu Gly Ala Asn Met Asn Ile Thr Thr Asn Asp Gly Asn Thr Cys						
		225		230		235
Gly Ser Phe Ala Val Met Asn Cys Asn Arg Asp Ile Asn Arg Leu Phe						
		245		250		255
Leu Asn Gln Asn Pro Asn Ile Glu Thr Ile Tyr Asn Thr Leu Lys Ile						
		260		265		270
Leu Ser Glu Asn Ile Val Phe Ile Asp Gly Cys Asp Val Arg Thr Asn						
		275		280		285
Met Val Lys Lys Ile Leu Met Tyr Gly Phe Thr Leu Asp Pro Leu Phe						
		290		295		300
Tyr Lys Asn His Asp Ile Ile Val Glu Tyr Phe Ser Ser Ser Ile Lys						
		305		310		315
Lys Tyr Asn Lys Ile Ile Leu Gln Met Ile Asp Glu Lys Ile Gly Asn						
		325		330		335
Arg Ser Val Tyr Asp Ile Ile Phe Thr Lys Ser Asn Thr Gly Met Asp						
		340		345		350
Val Arg Tyr Val Cys Asn Asp Ile Ile Ile Lys Tyr Ala Ser Val Lys						
		355		360		365
Tyr Tyr Gly Ser Leu Ile Lys Arg Leu Ile Tyr His Ser Lys Lys Arg						
		370		375		380
Lys Arg Asn Ile Leu Lys Ala Ile His Ala Met Glu Asn Asn Thr Thr						
		385		390		395
Leu Trp Asn Tyr Leu Pro Leu Glu Val Lys Met Tyr Ile Met Asp Phe						
		405		410		415
Leu Pro Asp Thr Asp Ile Thr Asn Ile Leu Phe Met Lys Lys						
		420		425		430

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 493 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asp Asn Leu Tyr Arg Tyr Ile Thr Val Ser Asp Thr Val Asp Val			
1	5	10	15

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Asp Asn Val Arg Lys Leu Leu Ser Ser Cys Asn Ile Asp Val Val Ser
 20 25 30
 Thr Ile Phe Gln Lys Tyr Leu His Arg Asn Asp Ile Lys Leu Asp Ile
 35 40 45
 Val Glu Glu Phe Val Asn Asn Gly Ala Lys Leu Asn Gly Lys Asp Phe
 50 55 60
 Asn Asp Lys Asn Ile Pro Leu Cys Thr Leu Leu Ser Asn Lys Phe Ile
 65 70 75 80
 Asp Tyr Asn Ser Ala Ile Asp Ile Thr Ser Phe Met Ile Thr His Gly
 85 90 95
 Ala Asp Ile Asn Lys Arg Asn Lys Asp Gly Arg Thr Pro Ile Phe Cys
 100 105 110
 Leu Leu His Asn Ser Thr Leu Asn Asn Leu Glu Phe Val Ser Phe Met
 115 120 125
 Ile Asp His Gly Ala Asp Ile Thr Ile Val Asp Gly Phe Gly Phe Thr
 130 135 140
 Ser Leu Gln Ile Tyr Leu Gln Ser Ser Asn Val Gln Leu Asp Leu Val
 145 150 155 160
 Glu Leu Leu Ile Gln Lys Gly Val Asp Val Asn Ile His Asn Asn Trp
 165 170 175
 Phe Tyr Tyr Asn Thr Leu His Cys Tyr Ile Lys Lys Asn Tyr Asn Arg
 180 185 190
 Ile Asn Met Asp Ile Ile Lys Tyr Ile Met Asp Asn Gly Phe Thr Ile
 195 200 205
 Asn Glu Asn Lys Phe Thr Lys Ser Thr Phe Leu Asp Ile Leu Val Ser
 210 215 220
 Ile Ile Asp Ser Lys Asn Phe Asp Ser Asn Val Val Asp Phe Ile Leu
 225 230 235 240
 Lys Tyr Ile Asp Ile Asn Glu Lys Asn Ile Phe Asp Phe Thr Pro Leu
 245 250 255
 Tyr Cys Ser Val Asp Ala Asn Asn Glu Lys Met Cys Ser Tyr Leu Leu
 260 265 270
 Lys Lys Asn Ala Asp Pro Asn Ile Ile Thr Val Phe Gly Glu Thr Cys
 275 280 285
 Ile Leu Thr Ala Ile Asn Asn His Asn Lys Asn Ile Leu Tyr Lys Leu
 290 295 300
 Leu Asn Tyr Asp Ile Asp Ile Asn Thr Ile Gln Asn Thr Leu Phe Lys
 305 310 315 320
 Leu Glu Gln Asp Ile Ile Asn Ser Thr Ile Asp Thr Tyr Tyr Tyr Asn
 325 330 335
 Asn Leu Val Lys Lys Glu His Phe Ile Lys Leu Phe Leu Ala Tyr Ile
 340 345 350
 Val Lys Arg Tyr Glu Lys Asn Ile Gly Ile Leu Phe Leu Asp Tyr Pro
 355 360 365
 Thr Leu Gly Glu Tyr Phe Val Lys Phe Ile Asp Thr Cys Met Met Glu

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370		375		380
Ile Phe Glu Met Lys Ser Asp Lys Ala Gly Asn Thr Asp Ile Tyr Ser				
385		390		395
				400
Ile Ile Phe Thr Asn Lys Tyr Ile Pro Ile Pro Tyr Ile Thr Cys Lys				
		405	410	415
Lys Leu Lys Lys Tyr Glu Ser Phe Val Val Tyr Gly Thr Glu Ile Lys				
		420	425	430
Ser Ile Ile Lys Ser Ser Lys Ile Arg Tyr Ala Ser Val Ile Lys Val				
		435	440	445
Thr Glu Tyr Ile Thr Ser Ile Cys Ser Glu Glu Thr Ser Leu Trp Asn				
		450	455	460
Ser Ile Pro Ile Glu Ile Lys His Lys Ile Ile Asn Asn Ile Asn Asn				
465		470	475	480
His Asp Met Tyr Ile Leu Tyr Lys Asn Arg Lys Lys Lys				
		485	490	

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3295 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Porcine Reproductive and Respiratory Virus
- (B) STRAIN: IA-2 Strain (NVSL)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 47..814

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 670..1431

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1215..1748

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1762..2361

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2349..2870

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2863..3231

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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GAATTCCCGG GCCCTGTCAT TGAACCAACT TTAGGCCTGA ATTGAAATGA AATGGGGTCC	60
ATGCAAAGCC TTTTGTGACAA AATTGGCCAA CTTTTTGTGG ATGCTTTCAC GGAGTTCTTG	120
GTGTCCATTG TTGATATCAT TATATTTTGT GCCATTTTGT TTGGCTTCAC CATCGCCGGT	180
TGGTTGGTGG TCTTTTGCAT CAGATTGGTT TGCTCCGCGA TACTCCGTAC GCGCCCTGCC	240
ATTCACTCTG AGCAATTACA GAAGATCTTA TGAAGCCTTT CTTTCCCAGT GCCAAGTGGG	300
CATTCCCACC TGGGGAACATA AACATCCTTT GGGGATGTTT TGGCACCATA AGGTGTCAAC	360
CCTGATTGAT GAGATGGTGT CGCGTCGAAT GTACCGCATC ATGGAAAAAG CAGGACAGGC	420
TGCCTGGAAA CAGGTGGTGA GCGAGGCTAC GCTGTCTCGC ATTAGTAGTT TGGATGTGGT	480
GGCTCATTTT CAGCATCTTG CCGCCATTGA AGCCGAGACC TGTAATATT TGGCCTCCCG	540
GCTGCCCATG CTACACAACC TGCGCATGAC AGGGTCAAAT GTAACCATAG TGTATAATAG	600
TACTTTGCAT CAGGTGTTTG CTATTTTTC AACCCTGGT TCCCGGCCAA AGCTTCATGA	660
TTTTCAGCAA TGGTTAATAG CTGTACATTC CTCCATATTT TCCTCTGTTG CAGCTTCTTG	720
TACTCTCTTT GTTGTGCTGT GGTTCGGGGT TCCAATACTA CGTACTGTTT TTGGTTTCCG	780
CTGGTTAGGG GCAATTTTTC TTTCGAACTC ACAGTGAATT ACACGGTGTG TCCACCTTGC	840
CTCACCCGGC AAGCAGCCGC AGAGGCCTAC GAACCCGGTA GGTCTCTTTG GTGCAGGATA	900
GGGTATGACC GATGTGGGGA GGACGATCAT GACGAGCTAG GGTTTATGGT ACCGTCTGGC	960
CTCTCCAGCG AAGGCCACTT GACCAGTGTT TACGCCTGGT TGGCGTTCTT GTCCTTCAGC	1020
TACACGGCCC AGTTCCATCC CGAGATATTC GGGATAGGGA ATGTGAGTCG AGTTTATGTT	1080
GACATCGAAC ATCAACTCAT CTGCGCCGAA CATGACGGGC AGAACACCAC CTTGCCTCGT	1140
CATGACAACA TTTCAGCCGT GTTTCAGACC TATTACCAAC ATCAAGTCGA CGGCGGCAAT	1200
TGGTTTCACC TAGAATGGCT GCGTCCCTTC TTTTCCTCAT GGTTGGTTTT AAATGTCTCT	1260
TGGTTTCTCA GCGGTTGCGC TGCAAACCAT GTTTCAGTTC GAGTCTTGCA GACATTAAGA	1320
CCAACACCAC CGCAGCGGCA AGCTTTGCTG TCCTCCAAGA CATCAGTTGC CTTAGGCATC	1380
GCAACTCGGC CTCTGAGGCG ATTCGCAAAA TCCCTCAGTG CCGTACGGCG ATAGGGACAC	1440
CCGTGTATAT TACCACCACA GCCAATGTGA CAGATGAGAA TTATTTACAT TCTTCTGATC	1500
TCCTCATGCT TTCTTCTTGC CTTTTCTATG CTTCTGAGAT GAGTGAAAAG GGATTTAAGG	1560
TGGTATTTGG CAATGTGTCA GGCATCGTGG CTGTGTGTGT CAATTTTACC AGCTACGTCC	1620
AACATGTCAG GGAGTTTACC CAACGCTCCT TGATGGTCGA CCATGTGCGG CTGCTCCATT	1680
TCATGACACC TGAGACCATG AGGTGGGCAA CTGTTTTAGC CTGTCTTTTT GCCATTCTGT	1740
TGGCAATTTG AATGTTTAAG TATGTTGGGG AAATGCTTGA CCGCGGGCTG TTGCTCGCGA	1800
TTGCTTTCTT TGTGGTGTAT CGTGCCGTTT TGTTTTGCTG TGCTCGTCAA CGCCAACAGC	1860
AACAGCAGCT CTCATCTACA GTTGATTAC AACTTGACGC TATGTGAGCT GAATGGCACA	1920
GATTGGCTAT CTAATAAATT TGATTGGGCA GTGGAGAGTT TTGTCATCTT TCCCGTTTTG	1980
ACTCACATTG TCTCCTATGG TGCCCTCACT ACCAGCCATT TCCTTGACAC AGTCGCTTTA	2040

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Pro Phe Thr Leu Ser Asn Tyr Arg Arg Ser Tyr Glu Ala Phe Leu Ser
 65 70 75 80
 Gln Cys Gln Val Asp Ile Pro Thr Trp Gly Thr Lys His Pro Leu Gly
 85 90 95
 Met Phe Trp His His Lys Val Ser Thr Leu Ile Asp Glu Met Val Ser
 100 105 110
 Arg Arg Met Tyr Arg Ile Met Glu Lys Ala Gly Gln Ala Ala Trp Lys
 115 120 125
 Gln Val Val Ser Glu Ala Thr Leu Ser Arg Ile Ser Ser Leu Asp Val
 130 135 140
 Val Ala His Phe Gln His Leu Ala Ala Ile Glu Ala Glu Thr Cys Lys
 145 150 155 160
 Tyr Leu Ala Ser Arg Leu Pro Met Leu His Asn Leu Arg Met Thr Gly
 165 170 175
 Ser Asn Val Thr Ile Val Tyr Asn Ser Thr Leu His Gln Val Phe Ala
 180 185 190
 Ile Phe Pro Thr Pro Gly Ser Arg Pro Lys Leu His Asp Phe Gln Gln
 195 200 205
 Trp Leu Ile Ala Val His Ser Ser Ile Phe Ser Ser Val Ala Ala Ser
 210 215 220
 Cys Thr Leu Phe Val Val Leu Trp Leu Arg Val Pro Ile Leu Arg Thr
 225 230 235 240
 Val Phe Gly Phe Arg Trp Leu Gly Ala Ile Phe Leu Ser Asn Ser Gln
 245 250 255

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 254 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Val Asn Ser Cys Thr Phe Leu His Ile Phe Leu Cys Cys Ser Phe
 1 5 10 15
 Leu Tyr Ser Leu Cys Cys Ala Val Val Ala Gly Ser Asn Thr Thr Tyr
 20 25 30
 Cys Phe Trp Phe Pro Leu Val Arg Gly Asn Phe Ser Phe Glu Leu Thr
 35 40 45
 Val Asn Tyr Thr Val Cys Pro Pro Cys Leu Thr Arg Gln Ala Ala Ala
 50 55 60
 Glu Ala Tyr Glu Pro Gly Arg Ser Leu Trp Cys Arg Ile Gly Tyr Asp
 65 70 75 80
 Arg Cys Gly Glu Asp Asp His Asp Glu Leu Gly Phe Met Val Pro Ser
 85 90 95
 Gly Leu Ser Ser Glu Gly His Leu Thr Ser Val Tyr Ala Trp Leu Ala

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100					105					110					
Phe	Leu	Ser	Phe	Ser	Tyr	Thr	Ala	Gln	Phe	His	Pro	Glu	Ile	Phe	Gly
		115					120					125			
Ile	Gly	Asn	Val	Ser	Arg	Val	Tyr	Val	Asp	Ile	Glu	His	Gln	Leu	Ile
	130					135					140				
Cys	Ala	Glu	His	Asp	Gly	Gln	Asn	Thr	Thr	Leu	Pro	Arg	His	Asp	Asn
145					150					155					160
Ile	Ser	Ala	Val	Phe	Gln	Thr	Tyr	Tyr	Gln	His	Gln	Val	Asp	Gly	Gly
				165					170					175	
Asn	Trp	Phe	His	Leu	Glu	Trp	Leu	Arg	Pro	Phe	Phe	Ser	Ser	Trp	Leu
			180					185					190		
Val	Leu	Asn	Val	Ser	Trp	Phe	Leu	Arg	Arg	Ser	Pro	Ala	Asn	His	Val
		195					200					205			
Ser	Val	Arg	Val	Leu	Gln	Thr	Leu	Arg	Pro	Thr	Pro	Pro	Gln	Arg	Gln
	210					215					220				
Ala	Leu	Leu	Ser	Ser	Lys	Thr	Ser	Val	Ala	Leu	Gly	Ile	Ala	Thr	Arg
225					230					235					240
Pro	Leu	Arg	Arg	Phe	Ala	Lys	Ser	Leu	Ser	Ala	Val	Arg	Arg		
				245				250							

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 178 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met	Ala	Ala	Ser	Leu	Leu	Phe	Leu	Met	Val	Gly	Phe	Lys	Cys	Leu	Leu
1				5				10						15	
Val	Ser	Gln	Ala	Phe	Ala	Cys	Lys	Pro	Cys	Phe	Ser	Ser	Ser	Leu	Ala
		20					25						30		
Asp	Ile	Lys	Thr	Asn	Thr	Thr	Ala	Ala	Ala	Ser	Phe	Ala	Val	Leu	Gln
		35					40					45			
Asp	Ile	Ser	Cys	Leu	Arg	His	Arg	Asn	Ser	Ala	Ser	Glu	Ala	Ile	Arg
	50					55				60					
Lys	Ile	Pro	Gln	Cys	Arg	Thr	Ala	Ile	Gly	Thr	Pro	Val	Tyr	Ile	Thr
	65				70				75					80	
Thr	Thr	Ala	Asn	Val	Thr	Asp	Glu	Asn	Tyr	Leu	His	Ser	Ser	Asp	Leu
			85					90						95	
Leu	Met	Leu	Ser	Ser	Cys	Leu	Phe	Tyr	Ala	Ser	Glu	Met	Ser	Glu	Lys
			100					105				110			
Gly	Phe	Lys	Val	Val	Phe	Gly	Asn	Val	Ser	Gly	Ile	Val	Ala	Val	Cys
		115					120					125			
Val	Asn	Phe	Thr	Ser	Tyr	Val	Gln	His	Val	Arg	Glu	Phe	Thr	Gln	Arg
	130						135				140				

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Ser Leu Met Val Asp His Val Arg Leu Leu His Phe Met Thr Pro Glu
 145 150 155 160
 Thr Met Arg Trp Ala Thr Val Leu Ala Cys Leu Phe Ala Ile Leu Leu
 165 170 175
 Ala Ile

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 200 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Leu Gly Lys Cys Leu Thr Ala Gly Cys Cys Ser Arg Leu Leu Ser
 1 5 10 15
 Leu Trp Cys Ile Val Pro Phe Cys Phe Ala Val Leu Val Asn Ala Asn
 20 25 30
 Ser Asn Ser Ser Ser His Leu Gln Leu Ile Tyr Asn Leu Thr Leu Cys
 35 40 45
 Glu Leu Asn Gly Thr Asp Trp Leu Ser Asn Lys Phe Asp Trp Ala Val
 50 55 60
 Glu Ser Phe Val Ile Phe Pro Val Leu Thr His Ile Val Ser Tyr Gly
 65 70 75 80
 Ala Leu Thr Thr Ser His Phe Leu Asp Thr Val Ala Leu Val Thr Val
 85 90 95
 Ser Thr Ala Gly Phe Val His Gly Arg Tyr Val Leu Ser Ser Ile Tyr
 100 105 110
 Ala Val Cys Ala Leu Ala Ala Leu Thr Cys Phe Val Ile Arg Phe Ala
 115 120 125
 Lys Asn Cys Met Ser Trp Arg Tyr Ser Cys Thr Arg Tyr Thr Asn Phe
 130 135 140
 Leu Leu Asp Thr Lys Gly Arg Leu Tyr Arg Trp Arg Ser Pro Val Ile
 145 150 155 160
 Ile Glu Lys Arg Gly Lys Val Glu Val Glu Gly His Leu Ile Asp Leu
 165 170 175
 Lys Arg Val Val Leu Asp Gly Ser Val Ala Thr Pro Ile Thr Arg Val
 180 185 190
 Ser Ala Glu Gln Trp Gly Arg Pro
 195 200

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 174 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```

Met Gly Ser Ser Leu Asp Asp Phe Cys Tyr Asp Ser Thr Ala Pro Gln
 1           5           10           15
Lys Val Leu Leu Ala Phe Ser Ile Thr Tyr Thr Pro Val Met Ile Tyr
          20           25           30
Ala Leu Lys Val Ser Arg Gly Arg Leu Leu Gly Leu Leu His Leu Leu
          35           40           45
Ile Phe Leu Asn Cys Ala Phe Thr Phe Gly Tyr Met Thr Phe Ala His
          50           55           60
Phe Gln Ser Thr Asn Lys Val Ala Leu Thr Met Gly Ala Val Val Ala
 65           70           75           80
Leu Leu Trp Gly Val Tyr Ser Ala Ile Glu Thr Trp Lys Phe Ile Thr
          85           90           95
Ser Arg Cys Arg Leu Cys Leu Leu Gly Arg Lys Tyr Ile Leu Ala Pro
          100          105          110
Ala His His Val Glu Ser Ala Ala Gly Phe His Pro Ile Ala Ala Asn
          115          120          125
Asp Asn His Ala Phe Val Val Arg Arg Pro Gly Ser Thr Thr Val Asn
          130          135          140
Gly Thr Leu Val Pro Gly Leu Lys Gly Leu Val Leu Gly Gly Arg Lys
          145          150          155          160
Ala Val Lys Gln Gly Val Val Asn Leu Val Lys Tyr Ala Lys
          165          170

```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 123 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```

Met Pro Asn Asn Asn Gly Lys Gln Gln Lys Arg Lys Lys Gly Asp Gly
 1           5           10           15
Gln Pro Val Asn Gln Leu Cys Gln Met Leu Gly Lys Ile Ile Ala Gln
          20           25           30
Gln Asn Gln Ser Arg Gly Lys Gly Pro Gly Lys Lys Asn Lys Lys Lys
          35           40           45
Asn Pro Glu Lys Pro His Phe Pro Leu Ala Thr Glu Asp Asp Val Arg
          50           55           60
His His Phe Thr Pro Ser Glu Arg Gln Leu Cys Leu Ser Ser Ile Gln
          65           70           75           80
Thr Ala Phe Asn Gln Gly Ala Gly Thr Cys Thr Leu Ser Asp Ser Gly
          85           90           95
Arg Ile Ser Tyr Thr Val Glu Phe Ser Leu Pro Thr His His Thr Val

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	100		105		110					
Arg	Leu	Ile	Arg	Val	Thr	Ala	Ser	Pro	Ser	Ala
	115						120			

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 37 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCGGATCCGG CGCGCCGGAT TTTCCTACAT CTACACT 37

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CTAAAATTGA ATTGTAAT 18

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 36 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TTGGCGCGCC CTAGATCTGT GTAGTTGATT GATTG 36

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TACGGCGCGC CGGGAAATGC TAAAGCCAAG CCCACA 36

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 42 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TTCGGATCCT GCTCAGACAG TATTGTGTAT GTTATCAAGA GC 42

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CCATGAATTC CTTCCCTGAA TGCAAGGAGG GCTTC 35

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CGGGATCCTC ACCCGGGCAG CGCGCTGTA 29

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CGGAATTCAC AAGGGCCGAC ATTGGCC 27

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ATCGGGATCC CGTTATTCTT CGCTGATGGT GG 32

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ATCGGAATTC GCGGTGCCTG TTGCTCTGGA TG 32

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CTTCGGATCC TCATGCCCCC CCGACGTCGG CCATC 35

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TCATGAATTC GGCCGCTCGC GGCGGTGCTG AACGC 35

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CGGGATCCCT AGGGCGCGGA GCCGAGGGC 29

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CGGAATTCAG GCCCGCTGGG GCGAGCGTGG 30

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

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CTTCGGATCC TCATGCCCCC CCGACGTCGG CCATC 35

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TCATGAATTC GGCCGCTCGC GCGGTGCTG AACGC 35

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CGGGATCCTT AATATAATTT TCTAGGTGCT AGTTG 35

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CGGAATTCGA TGAGTGATGG AGCAGTTCAA 30

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(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CGGGATCCTT AATATAATTT TCTAGGTGCT AGTTG 35

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CGGAATTCGA TGAGTGATGG AGCAGTTCAA 30

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CGGGATCCTT AATATAATTT TCTAGGTGCT AGTTG 35

(2) INFORMATION FOR SEQ ID NO:33:

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- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CGGAATTCTA TGTGTTTTTT TATAGGACTT 30

- (2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CGGGATCCTT AATATAATTT TCTAGGTGCT AGTTG 35

- (2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

CGGAATTCTA TGTGTTTTTT TATAGGACTT 30

- (2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 35 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CGTCAGATCT CAGGAGGTCA TAAGATGCCA TTAGC 35

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CGTTGAATTC GATGACTTGC CAGACTTACA ACTTG 35

(2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 33 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

CGTCGAATTC GATGTCTGGA GCCTCTAGTG GGA 33

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 base pairs

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- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CGTCGGATCC GGCTCAAATA GCCGATACTC TTCTT 35

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CGTCGAATTC AATGGAAAGT CCAACGCACC CAAAA 35

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 32 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CGTCGGATCC GGGGACTAAA TGGAATCATA CA 32

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 35 base pairs
 - (B) TYPE: nucleic acid

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(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CGGGAATTCG GGGTCGTCCT TAGATGACTT CTGCC 35

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 38 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GCGGATCCTT GTTATGTGGC ATATTTGACA AGGTTTAC 38

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

AATGAATTCG AAATGGGGTC CATGCAAAGC CTTTTTG 37

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CAAGGATCCC ACACCGTGTA ATTCAGTGTG AGTTCTG 36

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GTCGAATTCG CCAAATAACA ACGGCAAGCA GCAGAAG 37

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CAAGGATCCC AGCCCATCAT GCTGAGGGTG ATG 33

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 38 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

TTCGAATTCG GCTAATAGCT GTACATTCCT CCATATTT 38

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GGGGATCCTA TCGCCGTACG GCACTGAGGG 30

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

CCGAATTCGG CTGCGTCCCT TCTTTTCCTC ATGG 34

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

CTGGATCCTT CAAATTGCCA ACAGAATGGC AAAAAGAC 38

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

TTGAATTCGT TGGAGAAATG CTTGACCGCG GGC 33

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

GAAGGATCCT AAGGACGACC CCATTGTTCC GCTG 34

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

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(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

ATGAAGGCC TGTACCCCGT CACGA 25

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

CGGGATCCGG CTACAGGGCG TCGGGGTCCT C 31

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

CCGGATCCGG CGCGCGACGT GACCCGGCTC 30

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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225	230	235	240
Asn Met Phe Pro Ala Ile Ile Pro Ser Val Asn Asp Phe Ile Ser Thr			
	245	250	255
Val Val Asp Lys Asp Arg Leu Ile Asn Met Tyr Gly Ile Lys Cys Val			
	260	265	270
Ala Met Phe Ser Tyr Asp Ile Asn Met Ile Asp Leu Glu Ser Leu Asp			
	275	280	285
Asp Ser Asp Tyr Ile Phe Ile Glu Lys Asn Ile Ser Ile Tyr Asp Val			
	290	295	300
Lys Cys Arg Asp Phe Ala Asn Met Ile Arg Asp Lys Val Lys Arg Glu			
	305	310	315
			320
Lys Asn Arg Ile Leu Thr Thr Lys Cys Glu Asp Ile Ile Arg Tyr Ile			
	325	330	335
Lys Leu Phe Ser Lys Asn Arg Ile Asn Asp Glu Asn Asn Lys Val Glu			
	340	345	350
Glu Val Leu Ile His Ile Asp Asn Val Ser Lys Asn Asn Lys Leu Ser			
	355	360	365
Leu Ser Asp Ile Ser Ser Leu Met Asp Gln Phe Arg Leu Asn Pro Cys			
	370	375	380
Thr Ile Arg Asn Ile Leu Leu Ser Ser Ala Thr Ile Lys Ser Lys Leu			
	385	390	395
			400
Leu Ala Leu Arg Ala Val Lys Asn Trp Lys Cys Tyr Ser Leu Thr Asn			
	405	410	415
Val Ser Met Tyr Lys Lys Ile Lys Gly Val Ile Val Met Asp Met Val			
	420	425	430
Asp Tyr Ile Ser Thr Asn Ile Leu Lys Tyr His Lys Gln Leu Tyr Asp			
	435	440	445
Lys Met Ser Thr Phe Glu Tyr Lys Arg Asp Ile Lys Ser Cys Lys Cys			
	450	455	460
Ser Ile Cys Ser Asp Ser Ile Thr His His Ile Tyr Glu Thr Thr Ser			
	465	470	475
			480
Cys Ile Asn Tyr Lys Ser Thr Asp Asn Asp Leu Met Ile Val Leu Phe			
	485	490	495
Asn Leu Thr Arg Tyr Leu Met His Gly Met Ile His Pro Asn Leu Ile			
	500	505	510
Ser Val Lys Gly Trp Gly Pro Leu Ile Gly Leu Leu Thr Gly Asp Ile			
	515	520	525
Gly Ile Asn Leu Lys Leu Tyr Ser Thr Met Asn Ile Asn Gly Leu Arg			
	530	535	540
Tyr Gly Asp Ile Thr Leu Ser Ser Tyr Asp Met Ser Asn Lys Leu Val			
	545	550	555
			560
Ser Ile Ile Asn Thr Pro Ile Tyr Glu Leu Ile Pro Phe Thr Thr Cys			
	565	570	575
Cys Ser Leu Asn Glu Tyr Tyr Ser Lys Ile Val Ile Leu Ile Asn Val			
	580	585	590
Ile Leu Glu Tyr Met Ile Ser Ile Ile Leu Tyr Arg Ile Leu Ile Val			
	595	600	605
Lys Arg Phe Asn Asn Ile Lys Glu Phe Ile Ser Lys Val Val Asn Thr			
	610	615	620
Val Leu Glu Ser Ser Gly Ile Tyr Phe Cys Gln Met Arg Val His Glu			
	625	630	635
			640
Gln Ile Glu Leu Glu Ile Asp Glu Leu Ile Ile Asn Gly Ser Met Pro			

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(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

TCGAAGATCT TCTCATGCAA AGGTGGAACC GTTC 34

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

TCGAAGATCT CATGCCTATG TTCACCATCC ACAC 34

(2) INFORMATION FOR SEQ ID NO:189:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 3942 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

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(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Swinepox virus
- (B) STRAIN: Kasza
- (C) INDIVIDUAL ISOLATE: S-SPV-001

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..369

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 370..597

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 598..1539

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1675..3708

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: complement (3748..3942)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:189:

TGT	TTG	TTC	ATT	AAT	AAG	ATG	GGT	GGA	GCT	ATT	ATA	GAA	TAC	AAG	ATA	48
Cys	Leu	Phe	Ile	Asn	Lys	Met	Gly	Gly	Ala	Ile	Ile	Glu	Tyr	Lys	Ile	
1				5					10					15		
CCT	GGT	TCC	AAA	TCT	ATA	ACC	AAA	TCT	ATT	TCC	GAA	GAA	CTA	GAA	AAT	96
Pro	Gly	Ser	Lys	Ser	Ile	Thr	Lys	Ser	Ile	Ser	Glu	Glu	Leu	Glu	Asn	
			20					25					30			
TTA	ACA	AAG	CGA	GAT	AAA	CCA	ATA	TCT	AAA	ATT	ATA	GTT	ATT	CCT	ATT	144
Leu	Thr	Lys	Arg	Asp	Lys	Pro	Ile	Ser	Lys	Ile	Ile	Val	Ile	Pro	Ile	
		35					40					45				
GTA	TGT	TAC	AGA	AAT	GCA	AAT	AGT	ATA	AAG	GTT	ACA	TTT	GCA	CTA	AAA	192
Val	Cys	Tyr	Arg	Asn	Ala	Asn	Ser	Ile	Lys	Val	Thr	Phe	Ala	Leu	Lys	
	50					55				60						
AAG	TTT	ATC	ATA	GAT	AAG	GAG	TTT	AGT	ACA	AAT	GTA	ATA	GAC	GTA	GAT	240
Lys	Phe	Ile	Ile	Asp	Lys	Glu	Phe	Ser	Thr	Asn	Val	Ile	Asp	Val	Asp	
65				70				75						80		
GGT	AAA	CAT	GAA	AAA	ATG	TCC	ATG	AAT	GAA	ACA	TGC	GAA	GAG	GAT	GTT	288
Gly	Lys	His	Glu	Lys	Met	Ser	Met	Asn	Glu	Thr	Cys	Glu	Glu	Asp	Val	
				85				90						95		
GCT	AGA	GGA	TTG	GGA	ATT	ATA	GAT	CTT	GAA	GAT	GAA	TGC	ATA	GAG	GAA	336
Ala	Arg	Gly	Leu	Gly	Ile	Ile	Asp	Leu	Glu	Asp	Glu	Cys	Ile	Glu	Glu	
			100					105					110			
GAT	GAT	GTC	GAT	ACG	TCA	TTA	TTT	AAT	GTA	TAAATG	GAT	AAA	TTG	TAT		384
Asp	Asp	Val	Asp	Thr	Ser	Leu	Phe	Asn	Val	Met	Asp	Lys	Leu	Tyr		
		115					120			1				5		
GCG	GCA	ATA	TTC	GGC	GTT	TTT	ATG	ACA	TCT	AAA	GAT	GAT	GAT	TTT	AAT	432
Ala	Ala	Ile	Phe	Gly	Val	Phe	Met	Thr	Ser	Lys	Asp	Asp	Asp	Phe	Asn	
			10					15						20		
AAC	TTT	ATA	GAA	GTT	GTA	AAA	TCT	GTA	TTA	ACA	GAT	ACA	TCA	TCT	AAT	480

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Asn	Phe	Ile	Glu	Val	Val	Lys	Ser	Val	Leu	Thr	Asp	Thr	Ser	Ser	Asn	
			25					30					35			
CAT	ACA	ATA	TCG	TCG	TCC	AAT	AAT	AAT	ACA	TGG	ATA	TAT	ATA	TTT	CTA	528
His	Thr	Ile	Ser	Ser	Ser	Asn	Asn	Asn	Thr	Trp	Ile	Tyr	Ile	Phe	Leu	
		40				45					50					
GCG	ATA	TTA	TTT	GGT	GTT	ATG	GTA	TTA	TTA	GTT	TTT	ATT	TTG	TAT	TTA	576
Ala	Ile	Leu	Phe	Gly	Val	Met	Val	Leu	Leu	Val	Phe	Ile	Leu	Tyr	Leu	
		55				60				65						
AAA	GTT	ACT	AAA	CCA	ACT	TAAATG	GAG	GAA	GCA	GAT	AAC	CAA	CTC	GTT		624
Lys	Val	Thr	Lys	Pro	Thr	Met	Glu	Glu	Ala	Asp	Asn	Gln	Leu	Val		
		70			75	1				5						
TTA	AAT	AGT	ATT	AGT	GCT	AGA	GCA	TTA	AAG	GCA	TTT	TTT	GTA	TCT	AAA	672
Leu	Asn	Ser	Ile	Ser	Ala	Arg	Ala	Leu	Lys	Ala	Phe	Phe	Val	Ser	Lys	
		10			15					20					25	
ATT	AAT	GAT	ATG	GTC	GAT	GAA	TTA	GTT	ACC	AAA	AAA	TAT	CCA	CCA	AAG	720
Ile	Asn	Asp	Met	Val	Asp	Glu	Leu	Val	Thr	Lys	Lys	Tyr	Pro	Pro	Lys	
				30					35					40		
AAG	AAA	TCA	CAA	ATA	AAA	CTC	ATA	GAT	ACA	CGA	ATT	CCT	ATT	GAT	CTT	768
Lys	Lys	Ser	Gln	Ile	Lys	Leu	Ile	Asp	Thr	Arg	Ile	Pro	Ile	Asp	Leu	
			45					50				55				
ATT	AAT	CAA	CAA	TTC	GTT	AAA	AGA	TTT	AAA	CTA	GAA	AAT	TAT	AAA	AAT	816
Ile	Asn	Gln	Gln	Phe	Val	Lys	Arg	Phe	Lys	Leu	Glu	Asn	Tyr	Lys	Asn	
		60					65					70				
GGA	ATT	TTA	TCC	GTT	CTT	ATC	AAT	AGT	TTA	GTC	GAA	AAT	AAT	TAC	TTT	864
Gly	Ile	Leu	Ser	Val	Leu	Ile	Asn	Ser	Leu	Val	Glu	Asn	Asn	Tyr	Phe	
		75				80					85					
GAA	CAA	GAT	GGT	AAA	CTT	AAT	AGC	AGT	GAT	ATT	GAT	GAA	TTA	GTG	CTC	912
Glu	Gln	Asp	Gly	Lys	Leu	Asn	Ser	Ser	Asp	Ile	Asp	Glu	Leu	Val	Leu	
		90			95				100					105		
ACA	GAC	ATA	GAG	AAA	AAG	ATT	TTA	TCG	TTG	ATT	CCT	AGA	TGT	TCT	CCT	960
Thr	Asp	Ile	Glu	Lys	Lys	Ile	Leu	Ser	Leu	Ile	Pro	Arg	Cys	Ser	Pro	
				110				115					120			
CTT	TAT	ATA	GAT	ATC	AGT	GAC	GTT	AAA	GTT	CTC	GCA	TCT	AGG	TTA	AAA	1008
Leu	Tyr	Ile	Asp	Ile	Ser	Asp	Val	Lys	Val	Leu	Ala	Ser	Arg	Leu	Lys	
			125				130					135				
AAA	AGT	GCT	AAA	TCA	TTT	ACG	TTT	AAT	GAT	CAT	GAA	TAT	ATT	ATA	CAA	1056
Lys	Ser	Ala	Lys	Ser	Phe	Thr	Phe	Asn	Asp	His	Glu	Tyr	Ile	Ile	Gln	
		140					145					150				
TCT	GAT	AAA	ATA	GAG	GAA	TTA	ATA	AAT	AGT	TTA	TCT	AGA	AAC	CAT	GAT	1104
Ser	Asp	Lys	Ile	Glu	Glu	Leu	Ile	Asn	Ser	Leu	Ser	Arg	Asn	His	Asp	
		155				160					165					
ATT	ATA	CTA	GAT	GAA	AAA	AGT	TCT	ATT	AAA	GAC	AGC	ATA	TAT	ATA	CTA	1152
Ile	Ile	Leu	Asp	Glu	Lys	Ser	Ser	Ile	Lys	Asp	Ser	Ile	Tyr	Ile	Leu	
		170			175					180					185	
TCT	GAT	GAT	CTT	TTG	AAT	ATA	CTT	CGT	GAA	AGA	TTA	TTT	AGA	TGT	CCA	1200
Ser	Asp	Asp	Leu	Leu	Asn	Ile	Leu	Arg	Glu	Arg	Leu	Phe	Arg	Cys	Pro	
				190				195						200		
CAG	GTT	AAA	GAT	AAT	ACT	ATT	TCT	AGA	ACA	CGT	CTA	TAT	GAT	TAT	TTT	1248
Gln	Val	Lys	Asp	Asn	Thr	Ile	Ser	Arg	Thr	Arg	Leu	Tyr	Asp	Tyr	Phe	
			205				210						215			

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ACT AGA GTG TCA AAG AAA GAA GAA GCG AAA ATA TAC GTT ATA TTG AAA	1296
Thr Arg Val Ser Lys Lys Glu Glu Ala Lys Ile Tyr Val Ile Leu Lys	
220 225 230	
GAT TTA AAG ATT GCT GAT ATA CTC GGT ATC GAA ACA GTA ACG ATA GGA	1344
Asp Leu Lys Ile Ala Asp Ile Leu Gly Ile Glu Thr Val Thr Ile Gly	
235 240 245	
TCA TTT GTA TAT ACG AAA TAT AGC ATG TTG ATT AAT TCA ATT TCG TCT	1392
Ser Phe Val Tyr Thr Lys Tyr Ser Met Leu Ile Asn Ser Ile Ser Ser	
250 255 260 265	
AAT GTT GAT AGA TAT TCA AAA AGG TTC CAT GAC TCT TTT TAT GAA GAT	1440
Asn Val Asp Arg Tyr Ser Lys Arg Phe His Asp Ser Phe Tyr Glu Asp	
270 275 280	
ATT GCG GAA TTT ATA AAG GAT AAT GAA AAA ATT AAT GTA TCC AGA GTT	1488
Ile Ala Glu Phe Ile Lys Asp Asn Glu Lys Ile Asn Val Ser Arg Val	
285 290 295	
GTT GAA TGC CTT ATC GTA CCT AAT ATT AAT ATA GAG TTA TTA ACT GAA	1536
Val Glu Cys Leu Ile Val Pro Asn Ile Asn Ile Glu Leu Leu Thr Glu	
300 305 310	
TAAGTATATA TAAATGATTG TTTTATAAT GTTTGTTATC GCATTTAGTT TTGCTGTATG	1596
GTTATCATAT ACATTTTAA GGCCGTATAT GATAAATGAA AATATATAAG CACTTATTTT	1656
TGTTAGTATA ATAACACA ATG CCG TCG TAT ATG TAT CCG AAG AAC GCA AGA	1707
Met Pro Ser Tyr Met Tyr Pro Lys Asn Ala Arg	
1 5 10	
AAA GTA ATT TCA AAG ATT ATA TCA TTA CAA CTT GAT ATT AAA AAA CTT	1755
Lys Val Ile Ser Lys Ile Ile Ser Leu Gln Leu Asp Ile Lys Lys Leu	
15 20 25	
CCT AAA AAA TAT ATA AAT ACC ATG TTA GAA TTT GGT CTA CAT GGA AAT	1803
Pro Lys Lys Tyr Ile Asn Thr Met Leu Glu Phe Gly Leu His Gly Asn	
30 35 40	
CTA CCA GCT TGT ATG TAT AAA GAT GCC GTA TCA TAT GAT ATA AAT AAT	1851
Leu Pro Ala Cys Met Tyr Lys Asp Ala Val Ser Tyr Asp Ile Asn Asn	
45 50 55	
ATA AGA TTT TTA CCT TAT AAT TGT GTT ATG GTT AAA GAT TTA ATA AAT	1899
Ile Arg Phe Leu Pro Tyr Asn Cys Val Met Val Lys Asp Leu Ile Asn	
60 65 70 75	
GTT ATA AAA TCA TCA TCT GTA ATA GAT ACT AGA TTA CAT CAA TCT GTA	1947
Val Ile Lys Ser Ser Ser Val Ile Asp Thr Arg Leu His Gln Ser Val	
80 85 90	
TTA AAA CAT CGT AGA GCG TTA ATA GAT TAC GGC GAT CAA GAC ATT ATC	1995
Leu Lys His Arg Arg Ala Leu Ile Asp Tyr Gly Asp Gln Asp Ile Ile	
95 100 105	
ACT TTA ATG ATC ATT AAT AAG TTA CTA TCG ATA GAT GAT ATA TCC TAT	2043
Thr Leu Met Ile Ile Asn Lys Leu Leu Ser Ile Asp Asp Ile Ser Tyr	
110 115 120	
ATA TTA GAT AAA AAA ATA ATT CAT GTA ACA AAA ATA TTA AAA ATA GAC	2091
Ile Leu Asp Lys Lys Ile Ile His Val Thr Lys Ile Leu Lys Ile Asp	
125 130 135	
CCT ACA GTA GCC AAT TCA AAC ATG AAA CTG AAT AAG ATA GAG CTT GTA	2139
Pro Thr Val Ala Asn Ser Asn Met Lys Leu Asn Lys Ile Glu Leu Val	
140 145 150 155	

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GAT Asp	GTA Val	ATA Ile	ACA Thr	TCA Ser 160	ATA Ile	CCT Pro	AAG Lys	TCT Ser	TCC Ser 165	TAT Tyr	ACA Thr	TAT Tyr	TTA Leu	TAT Tyr 170	AAT Asn	2187
AAT Asn	ATG Met	ATC Ile	ATT Ile 175	GAT Asp	CTC Leu	GAT Asp	ACA Thr	TTA Leu 180	TTA Leu	TAT Tyr	TTA Leu	TCC Ser	GAT Asp 185	GCA Ala	TTC Phe	2235
CAC His	ATA Ile	CCC Pro 190	CCC Pro	ACA Thr	CAT His	ATA Ile	TCA Ser 195	TTA Leu	CGT Arg	TCA Ser	CTT Leu	AGA Arg 200	GAT Asp	ATA Ile	AAC Asn	2283
AGG Arg 205	ATT Ile	ATT Ile	GAA Glu	TTG Leu	CTT Leu	AAA Lys 210	AAA Lys	TAT Tyr	CCG Pro	AAT Asn 215	AAT Asn	AAT Asn	ATT Ile	ATT Ile	GAT Asp	2331
TAT Tyr 220	ATA Ile	TCC Ser	GAT Asp	AGC Ser	ATA Ile 225	AAA Lys	TCA Ser	AAT Asn	AGT Ser	TCA Ser 230	TTC Phe	ATT Ile	CAC His	ATA Ile	CTT Leu 235	2379
CAT His	ATG Met	ATA Ile	ATA Ile	TCA Ser 240	AAT Asn	ATG Met	TTT Phe	CCT Pro	GCT Ala 245	ATA Ile	ATC Ile	CCT Pro	AGT Ser	GTA Val 250	AAC Asn	2427
GAT Asp	TTT Phe	ATA Ile	TCT Ser 255	ACC Thr	GTA Val	GTT Val	GAT Asp 260	AAA Lys	GAT Asp	CGA Arg	CTT Leu	ATT Ile	AAT Asn 265	ATG Met	TAT Tyr	2475
GGG Gly	ATT Ile	AAG Lys 270	TGT Cys	GTT Val	GCT Ala	ATG Met	TTT Phe 275	TCG Ser	TAC Tyr	GAT Asp	ATA Ile	AAC Asn 280	ATG Met	ATC Ile	GAT Asp	2523
TTA Leu 285	GAG Glu	TCA Ser	TTA Leu	GAT Asp	GAC Asp	TCA Ser 290	GAT Asp	TAC Tyr	ATA Ile	TTT Phe 295	ATA Ile	GAA Glu	AAA Lys	AAT Asn	ATA Ile	2571
TCT Ser 300	ATA Ile	TAC Tyr	GAC Asp	GTT Val	AAA Lys 305	TGT Cys	AGA Arg	GAT Asp	TTT Phe	GCG Ala 310	AAT Asn	ATG Met	ATT Ile	AGA Arg	GAT Asp 315	2619
AAG Lys	GTT Val	AAA Lys	AGA Arg	GAA Glu 320	AAG Lys	AAT Asn	AGA Arg	ATA Ile	TTA Leu 325	ACT Thr	ACG Thr	AAA Lys	TGT Cys	GAA Glu 330	GAT Asp	2667
ATT Ile	ATA Ile	AGA Arg	TAT Tyr 335	ATA Ile	AAA Lys	TTA Leu	TTC Phe	AGT Ser 340	AAA Lys	AAT Asn	AGA Arg	ATA Ile	AAC Asn 345	GAT Asp	GAA Glu	2715
AAT Asn	AAT Asn	AAG Lys 350	GTG Val	GAG Glu	GAG Glu	GTG Val 355	TTG Leu	ATA Ile	CAT His	ATT Ile	GAT Asp	AAT Asn 360	GTA Val	TCT Ser	AAA Lys	2763
AAT Asn 365	AAT Asn	AAA Lys	TTA Leu	TCA Ser	CTG Leu	TCT Ser 370	GAT Asp	ATA Ile	TCA Ser	TCT Ser	TTA Leu 375	ATG Met	GAT Asp	CAA Gln	TTT Phe	2811
CGT Arg 380	TTA Leu	AAT Asn	CCA Pro	TGT Cys	ACC Thr 385	ATA Ile	AGA Arg	AAT Asn	ATA Ile	TTA Leu 390	TTA Leu	TCT Ser	TCA Ser	GCA Ala	ACT Thr 395	2859
ATA Ile	AAA Lys	TCA Ser	AAA Lys	CTA Leu 400	TTA Leu	GCG Ala	TTA Leu	CGG Arg	GCA Ala 405	GTA Val	AAA Lys	AAC Asn	TGG Trp	AAA Lys 410	TGT Cys	2907
TAT Tyr	TCA Ser	TTG Leu	ACA Thr 415	AAT Asn	GTA Val	TCA Ser	ATG Met	TAT Tyr	AAA Lys 420	AAA Lys	ATA Ile	AAG Lys	GGT Gly 425	GTT Val	ATC Ile	2955

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GTA ATG GAT ATG GTT GAT TAT ATA TCT ACT AAC ATT CTT AAA TAC CAT	3003
Val Met Asp Met Val Asp Tyr Ile Ser Thr Asn Ile Leu Lys Tyr His	
430 435 440	
AAA CAA TTA TAT GAT AAA ATG AGT ACG TTT GAA TAT AAA CGA GAT ATT	3051
Lys Gln Leu Tyr Asp Lys Met Ser Thr Phe Glu Tyr Lys Arg Asp Ile	
445 450 455	
AAA TCA TGT AAA TGC TCG ATA TGT TCC GAC TCT ATA ACA CAT CAT ATA	3099
Lys Ser Cys Lys Cys Ser Ile Cys Ser Asp Ser Ile Thr His His Ile	
460 465 470 475	
TAT GAA ACA ACA TCA TGT ATA AAT TAT AAA TCT ACC GAT AAT GAT CTT	3147
Tyr Glu Thr Thr Ser Cys Ile Asn Tyr Lys Ser Thr Asp Asn Asp Leu	
480 485 490	
ATG ATA GTA TTG TTC AAT CTA ACT AGA TAT TTA ATG CAT GGG ATG ATA	3195
Met Ile Val Leu Phe Asn Leu Thr Arg Tyr Leu Met His Gly Met Ile	
495 500 505	
CAT CCT AAT CTT ATA AGC GTA AAA GGA TGG GGT CCC CTT ATT GGA TTA	3243
His Pro Asn Leu Ile Ser Val Lys Gly Trp Gly Pro Leu Ile Gly Leu	
510 515 520	
TTA ACG GGT GAT ATA GGT ATT AAT TTA AAA CTA TAT TCC ACC ATG AAT	3291
Leu Thr Gly Asp Ile Gly Ile Asn Leu Lys Leu Tyr Ser Thr Met Asn	
525 530 535	
ATA AAT GGG CTA CGG TAT GGA GAT ATT ACG TTA TCT TCA TAC GAT ATG	3339
Ile Asn Gly Leu Arg Tyr Gly Asp Ile Thr Leu Ser Ser Tyr Asp Met	
540 545 550 555	
AGT AAT AAA TTA GTC TCT ATT ATT AAT ACA CCC ATA TAT GAG TTA ATA	3387
Ser Asn Lys Leu Val Ser Ile Ile Asn Thr Pro Ile Tyr Glu Leu Ile	
560 565 570	
CCG TTT ACT ACA TGT TGT TCA CTC AAT GAA TAT TAT TCA AAA ATT GTG	3435
Pro Phe Thr Thr Cys Cys Ser Leu Asn Glu Tyr Tyr Ser Lys Ile Val	
575 580 585	
ATT TTA ATA AAT GTT ATT TTA GAA TAT ATG ATA TCT ATT ATA TTA TAT	3483
Ile Leu Ile Asn Val Ile Leu Glu Tyr Met Ile Ser Ile Ile Leu Tyr	
590 595 600	
AGA ATA TTG ATC GTA AAA AGA TTT AAT AAC ATT AAA GAA TTT ATT TCA	3531
Arg Ile Leu Ile Val Lys Arg Phe Asn Asn Ile Lys Glu Phe Ile Ser	
605 610 615	
AAA GTC GTA AAT ACT GTA CTA GAA TCA TCA GGC ATA TAT TTT TGT CAG	3579
Lys Val Val Asn Thr Val Leu Glu Ser Ser Gly Ile Tyr Phe Cys Gln	
620 625 630 635	
ATG CGT GTA CAT GAA CAA ATT GAA TTG GAA ATA GAT GAG CTC ATT ATT	3627
Met Arg Val His Glu Gln Ile Glu Leu Met His Ile Asp Glu Leu Ile Ile	
640 645 650	
AAT GGA TCT ATG CCT GTA CAG CTT ATG CAT TTA CTT CTA AAG GTA GCT	3675
Asn Gly Ser Met Pro Val Gln Leu Met His Leu Leu Leu Lys Val Ala	
655 660 665	
ACC ATA ATA TTA GAG GAA ATC AAA GAA ATA TAACGTATTT TTTCTTTTAA	3725
Thr Ile Ile Leu Glu Glu Ile Lys Glu Ile	
670 675	
ATAAATAAAA ATACTTTTTT TTTTAAACAA GGGGTGCTAC CTTGTCTAAT TGTATCTTGT	3785
ATTTTGGATC TGATGCAAGA TTATTAAATA ATCGTATGAA AAAGTAGTAG ATATAGTTTA	3845

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TATCGTTACT GGACATGATA TTATGTTTAG TTAATTCCTC TTTGGCATGA ATTCTACACG 3905
 TCGGACAAGG TAATGTATCT ATAATGGTAT AAAGCTT 3942

(2) INFORMATION FOR SEQ ID NO:190:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 122 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:190:

Cys Leu Phe Ile Asn Lys Met Gly Gly Ala Ile Ile Glu Tyr Lys Ile
 1 5 10 15
 Pro Gly Ser Lys Ser Ile Thr Lys Ser Ile Ser Glu Glu Leu Glu Asn
 20 25 30
 Leu Thr Lys Arg Asp Lys Pro Ile Ser Lys Ile Ile Val Ile Pro Ile
 35 40 45
 Val Cys Tyr Arg Asn Ala Asn Ser Ile Lys Val Thr Phe Ala Leu Lys
 50 55 60
 Lys Phe Ile Ile Asp Lys Glu Phe Ser Thr Asn Val Ile Asp Val Asp
 65 70 75 80
 Gly Lys His Glu Lys Met Ser Met Asn Glu Thr Cys Glu Glu Asp Val
 85 90 95
 Ala Arg Gly Leu Gly Ile Ile Asp Leu Glu Asp Glu Cys Ile Glu Glu
 100 105 110
 Asp Asp Val Asp Thr Ser Leu Phe Asn Val
 115 120

(2) INFORMATION FOR SEQ ID NO:191:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 75 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:191:

Met Asp Lys Leu Tyr Ala Ala Ile Phe Gly Val Phe Met Thr Ser Lys
 1 5 10 15
 Asp Asp Asp Phe Asn Asn Phe Ile Glu Val Val Lys Ser Val Leu Thr
 20 25 30
 Asp Thr Ser Ser Asn His Thr Ile Ser Ser Ser Asn Asn Asn Thr Trp
 35 40 45
 Ile Tyr Ile Phe Leu Ala Ile Leu Phe Gly Val Met Val Leu Leu Val
 50 55 60
 Phe Ile Leu Tyr Leu Lys Val Thr Lys Pro Thr
 65 70 75

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(2) INFORMATION FOR SEQ ID NO:192:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 313 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:192:

Met	Glu	Glu	Ala	Asp	Asn	Gln	Leu	Val	Leu	Asn	Ser	Ile	Ser	Ala	Arg	
1				5			10							15		
Ala	Leu	Lys	Ala	Phe	Phe	Val	Ser	Lys	Ile	Asn	Asp	Met	Val	Asp	Glu	
			20					25					30			
Leu	Val	Thr	Lys	Lys	Tyr	Pro	Pro	Lys	Lys	Lys	Ser	Gln	Ile	Lys	Leu	
			35				40					45				
Ile	Asp	Thr	Arg	Ile	Pro	Ile	Asp	Leu	Ile	Asn	Gln	Gln	Phe	Val	Lys	
	50					55					60					
Arg	Phe	Lys	Leu	Glu	Asn	Tyr	Lys	Asn	Gly	Ile	Leu	Ser	Val	Leu	Ile	
	65				70				75						80	
Asn	Ser	Leu	Val	Glu	Asn	Asn	Tyr	Phe	Glu	Gln	Asp	Gly	Lys	Leu	Asn	
				85					90					95		
Ser	Ser	Asp	Ile	Asp	Glu	Leu	Val	Leu	Thr	Asp	Ile	Glu	Lys	Lys	Ile	
			100					105					110			
Leu	Ser	Leu	Ile	Pro	Arg	Cys	Ser	Pro	Leu	Tyr	Ile	Asp	Ile	Ser	Asp	
		115					120					125				
Val	Lys	Val	Leu	Ala	Ser	Arg	Leu	Lys	Lys	Ser	Ala	Lys	Ser	Phe	Thr	
	130					135					140					
Phe	Asn	Asp	His	Glu	Tyr	Ile	Ile	Gln	Ser	Asp	Lys	Ile	Glu	Glu	Leu	
	145				150					155					160	
Ile	Asn	Ser	Leu	Ser	Arg	Asn	His	Asp	Ile	Ile	Leu	Asp	Glu	Lys	Ser	
				165					170					175		
Ser	Ile	Lys	Asp	Ser	Ile	Tyr	Ile	Leu	Ser	Asp	Asp	Leu	Leu	Asn	Ile	
			180					185					190			
Leu	Arg	Glu	Arg	Leu	Phe	Arg	Cys	Pro	Gln	Val	Lys	Asp	Asn	Thr	Ile	
		195					200					205				
Ser	Arg	Thr	Arg	Leu	Tyr	Asp	Tyr	Phe	Thr	Arg	Val	Ser	Lys	Lys	Glu	
	210					215					220					
Glu	Ala	Lys	Ile	Tyr	Val	Ile	Leu	Lys	Asp	Leu	Lys	Ile	Ala	Asp	Ile	
	225				230					235					240	
Leu	Gly	Ile	Glu	Thr	Val	Thr	Ile	Gly	Ser	Phe	Val	Tyr	Thr	Lys	Tyr	
				245					250					255		
Ser	Met	Leu	Ile	Asn	Ser	Ile	Ser	Ser	Asn	Val	Asp	Arg	Tyr	Ser	Lys	
			260					265					270			
Arg	Phe	His	Asp	Ser	Phe	Tyr	Glu	Asp	Ile	Ala	Glu	Phe	Ile	Lys	Asp	
		275					280					285				

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Asn Glu Lys Ile Asn Val Ser Arg Val Val Glu Cys Leu Ile Val Pro
 290 295 300

Asn Ile Asn Ile Glu Leu Leu Thr Glu
 305 310

(2) INFORMATION FOR SEQ ID NO:193:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 677 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:193:

Met Pro Ser Tyr Met Tyr Pro Lys Asn Ala Arg Lys Val Ile Ser Lys
 1 5 10 15

Ile Ile Ser Leu Gln Leu Asp Ile Lys Lys Leu Pro Lys Lys Tyr Ile
 20 25 30

Asn Thr Met Leu Glu Phe Gly Leu His Gly Asn Leu Pro Ala Cys Met
 35 40 45

Tyr Lys Asp Ala Val Ser Tyr Asp Ile Asn Asn Ile Arg Phe Leu Pro
 50 55 60

Tyr Asn Cys Val Met Val Lys Asp Leu Ile Asn Val Ile Lys Ser Ser
 65 70 75 80

Ser Val Ile Asp Thr Arg Leu His Gln Ser Val Leu Lys His Arg Arg
 85 90 95

Ala Leu Ile Asp Tyr Gly Asp Gln Asp Ile Ile Thr Leu Met Ile Ile
 100 105 110

Asn Lys Leu Leu Ser Ile Asp Asp Ile Ser Tyr Ile Leu Asp Lys Lys
 115 120 125

Ile Ile His Val Thr Lys Ile Leu Lys Ile Asp Pro Thr Val Ala Asn
 130 135 140

Ser Asn Met Lys Leu Asn Lys Ile Glu Leu Val Asp Val Ile Thr Ser
 145 150 155 160

Ile Pro Lys Ser Ser Tyr Thr Tyr Leu Tyr Asn Asn Met Ile Ile Asp
 165 170 175

Leu Asp Thr Leu Leu Tyr Leu Ser Asp Ala Phe His Ile Pro Pro Thr
 180 185 190

His Ile Ser Leu Arg Ser Leu Arg Asp Ile Asn Arg Ile Ile Glu Leu
 195 200 205

Leu Lys Lys Tyr Pro Asn Asn Asn Ile Ile Asp Tyr Ile Ser Asp Ser
 210 215 220

Ile Lys Ser Asn Ser Ser Phe Ile His Ile Leu His Met Ile Ile Ser
 225 230 235 240

Asn Met Phe Pro Ala Ile Ile Pro Ser Val Asn Asp Phe Ile Ser Thr
 245 250 255

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Val Val Asp Lys Asp Arg Leu Ile Asn Met Tyr Gly Ile Lys Cys Val
260 265 270

Ala Met Phe Ser Tyr Asp Ile Asn Met Ile Asp Leu Glu Ser Leu Asp
275 280 285

Asp Ser Asp Tyr Ile Phe Ile Glu Lys Asn Ile Ser Ile Tyr Asp Val
290 295 300

Lys Cys Arg Asp Phe Ala Asn Met Ile Arg Asp Lys Val Lys Arg Glu
305 310 315 320

Lys Asn Arg Ile Leu Thr Thr Lys Cys Glu Asp Ile Ile Arg Tyr Ile
325 330 335

Lys Leu Phe Ser Lys Asn Arg Ile Asn Asp Glu Asn Asn Lys Val Glu
340 345 350

Glu Val Leu Ile His Ile Asp Asn Val Ser Lys Asn Asn Lys Leu Ser
355 360 365

Leu Ser Asp Ile Ser Ser Leu Met Asp Gln Phe Arg Leu Asn Pro Cys
370 375 380

Thr Ile Arg Asn Ile Leu Leu Ser Ser Ala Thr Ile Lys Ser Lys Leu
385 390 395 400

Leu Ala Leu Arg Ala Val Lys Asn Trp Lys Cys Tyr Ser Leu Thr Asn
405 410 415

Val Ser Met Tyr Lys Lys Ile Lys Gly Val Ile Val Met Asp Met Val
420 425 430

Asp Tyr Ile Ser Thr Asn Ile Leu Lys Tyr His Lys Gln Leu Tyr Asp
435 440 445

Lys Met Ser Thr Phe Glu Tyr Lys Arg Asp Ile Lys Ser Cys Lys Cys
450 455 460

Ser Ile Cys Ser Asp Ser Ile Thr His His Ile Tyr Glu Thr Thr Ser
465 470 475 480

Cys Ile Asn Tyr Lys Ser Thr Asp Asn Asp Leu Met Ile Val Leu Phe
485 490 495

Asn Leu Thr Arg Tyr Leu Met His Gly Met Ile His Pro Asn Leu Ile
500 505 510

Ser Val Lys Gly Trp Gly Pro Leu Ile Gly Leu Leu Thr Gly Asp Ile
515 520 525

Gly Ile Asn Leu Lys Leu Tyr Ser Thr Met Asn Ile Asn Gly Leu Arg
530 535 540

Tyr Gly Asp Ile Thr Leu Ser Ser Tyr Asp Met Ser Asn Lys Leu Val
545 550 555 560

Ser Ile Ile Asn Thr Pro Ile Tyr Glu Leu Ile Pro Phe Thr Thr Cys
565 570 575

Cys Ser Leu Asn Glu Tyr Tyr Ser Lys Ile Val Ile Leu Ile Asn Val
580 585 590

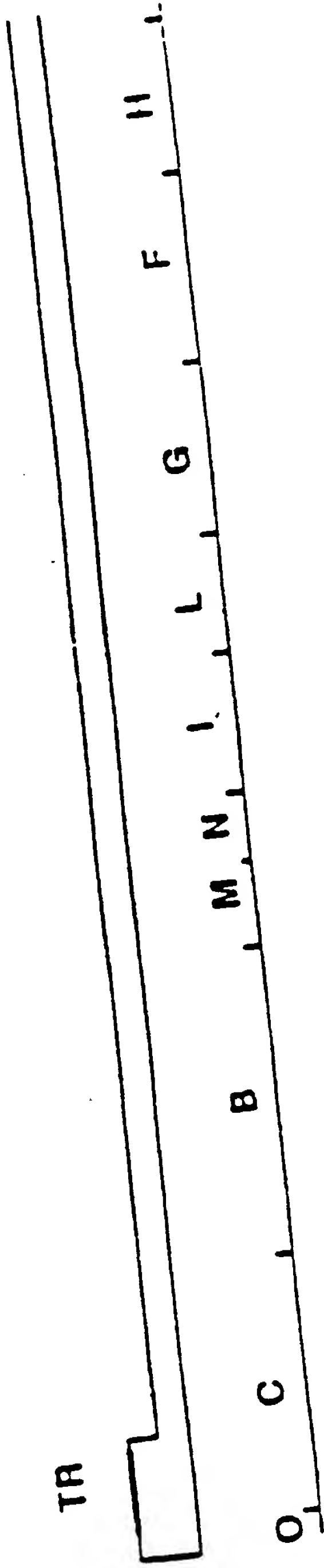
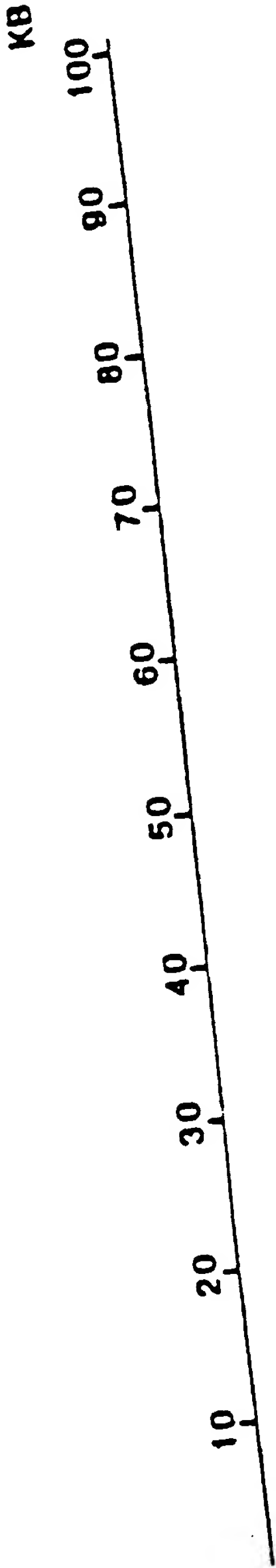
Ile Leu Glu Tyr Met Ile Ser Ile Ile Leu Tyr Arg Ile Leu Ile Val
595 600 605

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FIGURE 1A

FIGURE 1A	FIGURE 1B
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Lys Arg Phe Asn Asn Ile Lys Glu Phe Ile Ser Lys Val Val Asn Thr
 610 615 620
 Val Leu Glu Ser Ser Gly Ile Tyr Phe Cys Gln Met Arg Val His Glu
 625 630 635 640
 Gln Ile Glu Leu Glu Ile Asp Glu Leu Ile Ile Asn Gly Ser Met Pro
 645 650 655
 Val Gln Leu Met His Leu Leu Leu Lys Val Ala Thr Ile Ile Leu Glu
 660 665 670
 Glu Ile Lys Glu Ile
 675

(2) INFORMATION FOR SEQ ID NO:194:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 64 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:194:

Lys Leu Tyr Thr Ile Ile Asp Thr Leu Pro Cys Pro Thr Cys Arg Ile
 1 5 10 15
 His Ala Lys Glu Glu Leu Thr Lys His Asn Ile Met Ser Ser Asn Asp
 20 25 30
 Ile Asn Tyr Ile Tyr Tyr Phe Phe Ile Arg Leu Phe Asn Asn Leu Ala
 35 40 45
 Ser Asp Pro Lys Tyr Lys Ile Gln Leu Asp Lys Val Ala Pro Leu Val
 50 55 60

(2) INFORMATION FOR SEQ ID NO:195:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 583 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: Swinepox virus
 - (B) STRAIN: Kasza
 - (C) INDIVIDUAL ISOLATE: S-SPV-001

- (ix) FEATURE:
- (A) NAME/KEY: CDS
 - (B) LOCATION: 2..583

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:195:

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A AGC TTA AGA AAG AAT GTA GGG AAC GAA GAA TAT AGA ACC AAA GAT	46
Ser Leu Arg Lys Asn Val Gly Asn Glu Glu Tyr Arg Thr Lys Asp	
1 5 10 15	
TTA TTT ACT GCA TTA TGG GTA CCT GAT TTA TTT ATG GAA CGC GTA GAA	94
Leu Phe Thr Ala Leu Trp Val Pro Asp Leu Phe Met Glu Arg Val Glu	
20 25 30	
AAA GAT GAA GAA TGG TCT CTA ATG TGT CCA TGC GAA TGT CCA GGA TTA	142
Lys Asp Glu Glu Trp Ser Leu Met Cys Pro Cys Glu Cys Pro Gly Leu	
35 40 45	
TGC GAT GTA TGG GGG AAT GAT TTT AAC AAA TTA TAT ATA GAA TAC GAA	190
Cys Asp Val Trp Gly Asn Asp Phe Asn Lys Leu Tyr Ile Glu Tyr Glu	
50 55 60	
ACA AAG AAA AAA ATT AAA GCG ATC GCT AAA GCA AGA AGT TTA TGG AAA	238
Thr Lys Lys Lys Ile Lys Ala Ile Ala Lys Ala Arg Ser Leu Trp Lys	
65 70 75	
TCT ATT ATC GAG GCT CAA ATA GAA CAA GGA ACG CCG TAT ATA CTA TAT	286
Ser Ile Ile Glu Ala Gln Ile Glu Gln Gly Thr Pro Tyr Ile Leu Tyr	
80 85 90 95	
AAA GAT TCT TGT AAT AAA AAA TCC AAT CAA AGC AAT TTG GGA ACA ATT	334
Lys Asp Ser Cys Asn Lys Lys Ser Asn Gln Ser Asn Leu Gly Thr Ile	
100 105 110	
AGA TCG AGT AAT CTC TGT ACA GAG ATT ATA CAA TTT AGT AAC GAG GAT	382
Arg Ser Ser Asn Leu Cys Thr Glu Ile Ile Gln Phe Ser Asn Glu Asp	
115 120 125	
GAA GTT GCT GTA TGT AAT CTA GGA TCT ATT TCG TGG AGT AAA TTT GTT	430
Glu Val Ala Val Cys Asn Leu Gly Ser Ile Ser Trp Ser Lys Phe Val	
130 135 140	
AAT AAT AAC GTA TTT ATG TTC GAC AAG TTG AGA ATA ATT ACG AAA ATA	478
Asn Asn Asn Val Phe Met Phe Asp Lys Leu Arg Ile Ile Thr Lys Ile	
145 150 155	
CTA GTT AAA AAT CTA AAT AAA ATA ATA GAT ATC AAT TAT TAT CCA GTG	526
Leu Val Lys Asn Leu Asn Lys Ile Ile Asp Ile Asn Tyr Tyr Pro Val	
160 165 170 175	
ATA GAA TCG TCT AGA TCT AAT AAG AAA CAT AGA CCC ATA GGT ATC GGG	574
Ile Glu Ser Ser Arg Ser Asn Lys Lys His Arg Pro Ile Gly Ile Gly	
180 185 190	
GTT CAG GGT	583
Val Gln Gly	

(2) INFORMATION FOR SEQ ID NO:196:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 194 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:196:

Ser Leu Arg Lys Asn Val Gly Asn Glu Glu Tyr Arg Thr Lys Asp Leu
1 5 10 15

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Phe Thr Ala Leu Trp Val Pro Asp Leu Phe Met Glu Arg Val Glu Lys
20 25 30
Asp Glu Glu Trp Ser Leu Met Cys Pro Cys Glu Cys Pro Gly Leu Cys
35 40 45
Asp Val Trp Gly Asn Asp Phe Asn Lys Leu Tyr Ile Glu Tyr Glu Thr
50 55 60
Lys Lys Lys Ile Lys Ala Ile Ala Lys Ala Arg Ser Leu Trp Lys Ser
65 70 75 80
Ile Ile Glu Ala Gln Ile Glu Gln Gly Thr Pro Tyr Ile Leu Tyr Lys
85 90 95
Asp Ser Cys Asn Lys Lys Ser Asn Gln Ser Asn Leu Gly Thr Ile Arg
100 105 110
Ser Ser Asn Leu Cys Thr Glu Ile Ile Gln Phe Ser Asn Glu Asp Glu
115 120 125
Val Ala Val Cys Asn Leu Gly Ser Ile Ser Trp Ser Lys Phe Val Asn
130 135 140
Asn Asn Val Phe Met Phe Asp Lys Leu Arg Ile Ile Thr Lys Ile Leu
145 150 155 160
Val Lys Asn Leu Asn Lys Ile Ile Asp Ile Asn Tyr Tyr Pro Val Ile
165 170 175
Glu Ser Ser Arg Ser Asn Lys Lys His Arg Pro Ile Gly Ile Gly Val
180 185 190
Gln Gly

(2) INFORMATION FOR SEQ ID NO:197:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:197:

ACAGGAAACA GCTATGACCA TGATTACGAA TTCGAGCTCG CCCGGGGATC T

51

(2) INFORMATION FOR SEQ ID NO:198:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 138 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:198:

GTATAGCGGC CGCCTGCAGG TCGACTCTAG ATTTTTTTTT TTTTTTTTTT TGGCATATAA	60
ATAGATCTGT ATCCTAAAAT TGAATTGTAA TTATCGATAA TAAATGAATT CGATGGCTGT	120
GCCTGCAAGC CCACAGCA	138

(2) INFORMATION FOR SEQ ID NO:199:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:199:

CTTAGCCCCA AACGCACCTC AGATCCATAA TTAATTAATT TTTATCCCGG CGCGCCTCGA	60
CTCTAGAATT TCATTTTGTT TTTTCTATG CTATAAATGA ATTCGGATCC CGTCGTTTTA	120

(2) INFORMATION FOR SEQ ID NO:200:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 116 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:200:

GAAATCCAGC TGAGCGCCGG TCGTACCAT TACCAGTTGG TCTGGTGTCA AAAAGATCCA	60
TAATTAATTA ACCCGGGTCG AGGCGCGCCG GGTCGACCTG CAGGCGGCCG CTATAC	116

(2) INFORMATION FOR SEQ ID NO:201:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:201:

TAATGTATCT ATAATGGTAT AAAGCTTGTA TTCTATAGTG TCACCTAAAT C

51

(2) INFORMATION FOR SEQ ID NO:202:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 51 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:202:

ACAGGAAACA GCTATGACCA TGATTACGAA TTCGAGCTCG CCCGGGGATC T

51

(2) INFORMATION FOR SEQ ID NO:203:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 141 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:203:

GTATAGCGGC CGCCTGCAGG TCGACTCTAG ATTTTTTTTT TTTTTTTTTT TGGCATATAA 60

ATAGATCTGT ATCCTAAAAT TGAATTGTAA TTATCGATAA TAAATGAATT CCATGTGCTG 120

CCTCACCCCT GTGCTGGCGC T 141

(2) INFORMATION FOR SEQ ID NO:204:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 120 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:204:

TCGCCCCGCCT CTGACGCCCC GGATCCATAA TTAATTAATT TTTATCCCGG CGCGCCTCGA 60
CTCTAGAATT TCATTTTGTT TTTTCTATG CTATAAATGA ATTCGGATCC CGTCGTTTTA 120

(2) INFORMATION FOR SEQ ID NO:205:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 116 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:205:

GAAATCCAGC TGAGCGCCGG TCGCTACCAT TACCAGTTGG TCTGGTGTCA AAAAGATCCA 60
TAATTAATTA ACCCGGGTCG AGGCGCGCCG GGTCGACCTG CAGGCGGCCG CTATAC 116

(2) INFORMATION FOR SEQ ID NO:206:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:206:

TAATGTATCT ATAATGGTAT AAAGCTTGTA TTCTATAGTG TCACCTAAAT C 51

(2) INFORMATION FOR SEQ ID NO:207:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

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(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:207:

CAAGGAATGG TGCATGCCCG TTCTTATCAA TAGTTTAGTC GAAAA 45

(2) INFORMATION FOR SEQ ID NO:208:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 57 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:208:

TATATAAGCA CTTATTTTTG TTAGTATAAT AACACAATGC CAGATCCCGT CGTTTTA 57

(2) INFORMATION FOR SEQ ID NO:209:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 249 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:209:

TCCAGCTGAG CGCCGGTCGC TACCATTACC AGTTGGTCTG GTGTCAAAAA GATCCATAAT 60
 TAATTAACCA GCGGCCGCCT GCAGGTCGAC TCTAGATTTT TTTTTTTTTT TTTTTTGGCA 120
 TATAAATAGA TCTGTATCCT AAAATTGAAT TGTAATTATC GATAATAAAT GAATTCGGAT 180
 CCATAATTAA TTAATTTTTA TCCCGGCGCG CCGGGTCGAC CTGCAGGCGG CCGCTGGGTC 240
 GACAAAGAT 249

(2) INFORMATION FOR SEQ ID NO:210:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 45 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:210:

CAAAAGTCGT AAATACTGTA CTAGAAGCTT GCGTAATCA TGGTC

45

(2) INFORMATION FOR SEQ ID NO:211:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:211:

CGACGGATCC GAGGTGCGTT TGGGGCTAAG TGC

33

(2) INFORMATION FOR SEQ ID NO:212:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:212:

CCACGGATCC AGCACAACGC GAGTCCCACC ATGGCT

36

(2) INFORMATION FOR SEQ ID NO:213:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:213:

CCACGAATTC GATGGCTGTG CCTGCAAGCC CACAG

35

(2) INFORMATION FOR SEQ ID NO:214:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:214:

CGAAGATCTG AGGTGCGTTT GGGGCTAAGT GC

32

(2) INFORMATION FOR SEQ ID NO:215:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:215:

CGCAGGATCC GGGGCGTCAG AGGCGGGCGA GGTG

34

(2) INFORMATION FOR SEQ ID NO:216:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:216:

GAGCGGATCC TGCAGGAGGA GACACAGAGC TG

32

(2) INFORMATION FOR SEQ ID NO:217:

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- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 32 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:217:

GCGCGAATTC CATGTGCTGC CTCACCCCTG TG

32

(2) INFORMATION FOR SEQ ID NO:218:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 34 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:218:

CGCAGGATCC GGGGCGTCAG AGGCGGGCGA GGTG

34

(2) INFORMATION FOR SEQ ID NO:219:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 32 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:219:

GGGGAATTCA ATGCAACCCA CCGCGCCGCC CC

32

(2) INFORMATION FOR SEQ ID NO:220:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 32 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:220:

GGGGATCCT AGGGCGCGCC CGCCGGCTCG CT

32

(2) INFORMATION FOR SEQ ID NO:221:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5785 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:221:

AAGCTTAAGA AAGAATGTAG GGAACGAAGA ATATAGAACC AAAGATTTAT TTACTGCATT
60ATGGGTACCT GATTTATTTA TGGAACGCGT AGAAAAAGAT GAAGAATGGT CTCTAATGTG
120TCCATGCGAA TGTCCAGGAT TATGCGATGT ATGGGGGAAT GATTTTAACA AATTATATAT
180AGAATACGAA ACAAGAAAA AAATTAAAGC GATCGCTAAA GCAAGAAGTT TATGGAAATC
240TATTATCGAG GCTCAAATAG AACAAGGAAC GCCGTATATA CTATATAAAG ATTCTTGTA
300TAAAAAATCC AATCAAAGCA ATTTGGGAAC AATTAGATCG AGTAATCTCT GTACAGAGAT
360TATACAATTT AGTAACGAGG ATGAAGTTGC TGTATGTAAT CTAGGATCTA TTTCGTGGAG
420TAAATTTGTT AATAATAACG TATTTATGTT CGACAAGTTG AGAATAATTA CGAAAATACT
480AGTTAAAAAT CTAAATAAAA TAATAGATAT CAATTATTAT CCAGTGATAG AATCGTCTAG
540ATCTAATAAG AACATAGAC CCATAGGTAT CGGTGTTTCAG GGTTCGGCTG ATGTGTTTAT
600ATTATTGGGC TATGCATTCTG ATAGCGAAGA AGCAAAAATA TTAAATATAC AAATTTCCGA
660AACAAATATAT TATGCCGCAC TAGAATCTAG TTGCGAACTA GCTAAAATTT ACGGACCTTA
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TGAGACATAT AACGATTCTC CAGCGAGTAA AGGTATTCTA CAATATGATA TGTGGTTAAA
780

GAACCCAACA GATTTATGGG ATTGGAATGA ACTAAAAAG AGAATTAATA CACATGGATT
840

GAGAAATAGC CTTCTAATAG CACCAATGCC TACTGCATCT ACATCTCAA TATTAAGTAA
900

TAATGAGTCC ACCGAACCAT AACTAGCAA TATATATACA AGAAGAGTAT TATCTGGAGA
960

TTTTCAGGTT GTAAATCCAC ACCTATTGAG AGAACTAATA AGTAGAAATA TGTGGAATAA
1020

TGACATAAAG AATACAATTG TGTTACATAA TGGTTCTATT CAACATTTAG ATTTACCAGA
1080

TAATATAAAA CCAATATATA AAACGGTTTG GGAGATATCT CCAAAATGTA TTTTAGAAAT
1140

GGCAGCCGAC AGAGGTGCGT TTATAGATCC AAGTCAATCA ATGACAATAT ATATAGATAA
1200

TCCTACATAC GCAAACTGA CCAGTATGCA TTTTACGGA TGGAGATTGG GGCTAAAAAC
1260

TGGGATGTAT TATATGAGAA CAAATCGGC ATCAAATCCT ATAAATTCA CAGTTGAGTG
1320

TAGTAATTGT TCTGCATAAT TTTTATAAAA ATGAAATACT ATCTCATGTA TCTTAATATA
1380

TTAAAAATGC GTAAAAGTGG CATTCCAAA CAACCCGTTT CCAAAAAGA TTATGTTCAA
1440

ACTGATAATA ATAAAAACA ACAATAACA ACGTGTTTCA AAGTCGTTGA GTATCTTAAA
1500

TCACCTAAGTA AGAGCACCGA AAAATGTATA GAAAATGTAA TATTAACGCC TTCTCAATAT
1560

CCTTCTTGTT CATCGATAAC TATTAATTTA ACAGACTATC TATCATCTAA AATGACATCT
1620

ACATATATAG CATTAGAAGG TGAGTCTAAA ATATACAAGA ATAAAAAGAA TGAAAGTAGA
1680

TCGTTAGATC AATATTTTTT AAAAATACGA CTTACTGCAG CAAGTCCTAT AATGTATCAA
1740

TTATTAGATT GTATATATTC TAATATTAGA GATAATAAAC ATATACCCCC TTCCTTATCA
1800

AATATATCTA TATCGGACTT AGAAGAGAAA ACGCTTAACC AGGGGTGTTT GTTCATTAAT
1860

AAGATGGGTG GAGCTATTAT AGAATACAAG ATACCTGGTT CCAAATCTAT AACAAAATCT
1920

ATTTCCGAAG AACTAGAAAA TTTAACAAAG CGAGATAAAC AAATATCTAA AATTATAGTT
1980

ATTCCTATTG TATGTTACAG AAATGCAAAT AGTATAAAGG TTACATTGTC ACTAAAAAG
2040

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TTTATCATAG ATAAGGAGTT TAGTACAAAT GTAATAGACG TAGATGGTAA ACATGAAAAA
2100

ATGTCCATGA ATGAAACATG CGAAGAGGAT GTTGCTAGAG GATTGGGAAT TATAGATCTT
2160

GAAGATGAAT GCATAGAGGA AGATGATGTC GATACGTCAT TATTTAATGT ATAAATGGAT
2220

AAATTGTATG CGGCAATATT CGGCGTTTTT ATGACATCTA AAGATGATGA TTTTAATAAC
2280

TTTATAGAAG TTGTAAAATC TGTATTAACA GATACATCAT CTAATCATAC AATATCGTCG
2340

TCCAATAATA ATACATGGAT ATATATATTT CTAGCGATAT TATTTGGTGT TATGGTATTA
2400

TTAGTTTTTA TTTTGTATTT AAAAGTTACT AAACCAACTT AAATGGAGGA AGCAGATAAC
2460

CAACTCGTTT TAAATAGTAT TAGTGCTAGA GCATTAAAGG CATTTTTTGT ATCTAAAATT
2520

AATGATATGG TCGATGAATT AGTTACCAA AAATATCCAC CAAAGAAGAA ATCACAAATA
2580

AAACTCATAG ATACACGAAT TCCTATTGAT CTTATTAATC AACAATTCGT TAAAAGATTT
2640

AAACTAGAAA ATTATAAAAA TGAATTTTA TCCGTTCTTA TCAATAGTTT AGTCGAAAAT
2700

AATTACTTTG AACAAGATGG TAAACTTAAT AGCAGTGATA TTGATGAATT AGTGCTCACA
2760

GACATAGAGA AAAAGATTTT ATCGTTGATT CCTAGATGTT CTCCTCTTTA TATAGATATC
2820

AGTGACGTTA AAGTTCTCGC ATCTAGGTTA AAAAAGTGCT AAATCATTTA CGTTTAATGA
2880

TCATGAATAT ATTATACAAT CTGATAAAAT AGAGGAATTA ATAAATAGTT TATCTAGAAA
2940

CCATGATATT ATACTAGATG AAAAAAGTTC TATTAAAGAC AGCATATATA TACTATCTGA
3000

TGATCTTTTG AATATACTTC GTGAAAGATT ATTTAGATGT CCACAGGTTA AAGATAATAC
3060

TATTTCTAGA ACACGTCTAT ATGATTATTT TACTAGAGTG TCAAAGAAAG AAGAAGCGAA
3120

AATATACGTT ATATTGAAAG ATTTAAAGAT TGCTGATATA CTCGGTATCG AAACAGTAAC
3180

GATAGGATCA TTTGTATATA CGAAATATAG CATGTTGATT AATTCAATTT CGTCTAATGT
3240

TGATAGATAT TCAAAAAGGT TCCATGACTC TTTTATGAA GATATTGCGG AATTTATAAA
3300

GGATAATGAA AAAATTAATG TATCCAGAGT TGTTGAATGC CTTATCGTAC CTAATATTAA
3360

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TATAGAGTTA TTAAGTGAAT AAGTATATAT AAATGATTGT TTTTATAATG TTTGTTATCG
3420

CATTTAGTTT TGCTGTATGG TTATCATATA CATTTTTAAG GCCGTATATG ATAAATGAAA
3480

ATATATAAGC ACTTATTTTT GTTAGTATAA TAACACAATG CCGTCGTATA TGTATCCGAA
3540

GAACGCAAGA AAAGTAATTT CAAAGATTAT ATCATTACAA CTTGATATTA AAAAAGTTCC
3600

TAAAAAATAT ATAAATACCA TGTTAGAATT TGGTCTACAT GGAAATCTAC CAGCTTGTAT
3660

GTATAAAGAT GCCGTATCAT ATGATATAAA TAATATAAGA TTTTACCTT ATAATTGTGT
3720

TATGGTTAAA GATTTAATAA ATGTTATAAA ATCATCATCT GTAATAGATA CTAGATTACA
3780

TCAATCTGTA TTAAACATC GTAGAGCGTT AATAGATTAC GGCGATCAAG ACATTATCAC
3840

TTTAATGATC ATTAATAAGT TACTATCGAT AGATGATATA TCCTATATAT TAGATAAAAA
3900

AATAATTCAT GTAACAAAAA TATTAAAAAT AGACCCTACA GTAGCCAATT CAAACATGAA
3960

ACTGAATAAG ATAGAGCTTG TAGATGTAAT AACATCAATA CCTAAGTCTT CCTATACATA
4020

TTTATATAAT AATATGATCA TTGATCTCGA TACATTATTA TATTTATCCG ATGCATTCCA
4080

CATACCCCCC ACACATATAT CATTACGTTT ACTTAGAGAT ATAAACAGGA TTATTGAATT
4140

GCTTAAAAAA TATCCGAATA ATAATATTAT TGATTATATA TCCGATAGCA TAAATCAAA
4200

TAGTTCATTC ATTCACATAC TTCATATGAT AATATCAAAT ATGTTTCCTG CTATAATCCC
4260

TAGTGTAAAC GATTTTATAT CTACCGTAGT TGATAAAGAT CGACTTATTA ATATGTATGG
4320

GATTAAGTGT GTTGCTATGT TTTCGTACGA TATAAACATG ATCGATTAG AGTCATTAGA
4380

TGACTCAGAT TACATATTTA TAGAAAAAAA TATATCTATA TACGACGTTA AATGTAGAGA
4440

TTTTGCGAAT ATGATTAGAG ATAAGGTTAA AAGAGAAAAG AATAGAATAT TAACTACGAA
4500

ATGTGAAGAT ATTATAAGAT ATATAAAATT ATTCAGTAAA AATAGAATAA ACGATGAAAA
4560

TAATAAGGTG GAGGAGGTGT TGATACATAT TGATAATGTA TCTAAAAATA ATAAATTATC
4620

ACTGTCTGAT ATATCATCTT TAATGGATCA ATTTTCGTTT AATCCATGTA CCATAAGAAA
4680

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TATATTATTA TCTTCAGCAA CTATAAAATC AAAACTATTA GCGTTACGGG CAGTAAAAAA
4740

CTGGAAATGT TATTCATTGA CAAATGTATC AATGTATAAA AAAATAAAGG GTGTTATCGT
4800

AATGGATATG GTTGATTATA TATCTACTAA CATTCTTAAA TACCATAAAC AATTATATGA
4860

TAAATGAGT ACGTTTGAAT ATAAACGAGA TATTAAATCA TGTAATGCT CGATATGTTC
4920

CGACTCTATA ACACATCATA TATATGAAAC AACATCATGT ATAAATTATA AATCTACCGA
4980

TAATGATCTT ATGATAGTAT TGTTCATCT AACTAGATAT TTAATGCATG GGATGATACA
5040

TCCTAATCTT ATAAGCGTAA AAGGATGGGG TCCCCTTATT GGATTATTAA CGGGTGATAT
5100

AGGTATTAAT TTAAACTAT ATTCCACCAT GAATATAAAT GGGCTACGGT ATGGAGATAT
5160

TACGTTATCT TCATACGATA TGAGTAATAA ATTAGTCTCT ATTATTAATA CACCCATATA
5220

TGAGTTAATA CCGTTTACTA CATGTTGTTC ACTCAATGAA TATTATTCAA AAATTGTGAT
5280

TTTAATAAAT GTTATTTTAG AATATATGAT ATCTATTATA TTATATAGAA TATTGATCGT
5340

AAAAAGATTT AATAACATTA AAGAATTTAT TTCAAAGTC GTAAATACTG TACTAGAATC.
5400

ATCAGGCATA TATTTTTGTC AGATGCGTGT ACATGAACAA ATTGAATTGG AAATAGATGA
5460

GCTCATTATT AATGGATCTA TGCCTGTACA GCTTATGCAT TTACTTCTAA AGGTAGCTAC
5520

CATAATATTA GAGGAAATCA AAGAAATATA ACGTATTTTT TCTTTTAAAT AAATAAAAAT
5580

ACTTTTTTTT TTAAACAAGG GGTGCTACCT TGTCTAATTG TATCTTGTAT TTTGGATCTG
5640

ATGCAAGATT ATTAAATAAT CGTATGAAAA AGTAGTAGAT ATAGTTTATA TCGTTACTGG
5700

ACATGATATT ATGTTTAGTT AATTCTTCTT TGGCATGAAT TCTACACGTC GGACAAGGTA
5760

ATGTATCTAT AATGGTATAA AGCTT
5785

(2) INFORMATION FOR SEQ ID NO:222:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 722 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

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(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:222:

TTTTGATTTT ACGCCATTAT ACTGTTCTGT AGATGCAAAT AATGAAGATG TGTTCTTATT
60

TACTAGAGAG ATGCAGACCC TATATTATCA CAGTATTTGG TGAACGTGTA TACTAACAGC
120

TTCAATAATC ATAATCCCCC ATATTATATA ACTATTAAAT TATGATATAG ATATAAATAC
180

TATCCAAAAT ACATTATTTA AACTGGAACA AGATATTATT AACTCTACCA TAGATACTTA
240

CTATTACAAT AATCTTGTTA AAAAAGAACA TTTTATAAAA TTATTTCTAG CCTACATAGT
300

TAAGAGGTAT GAAAAAATA TAGGAATATT ATTTCTTGAT TATCCCACTC TTGGTGAATA
360

TTTCGTGAAA TTTATAGATA CGTGTATGAT GGAAATATTT GAGATGAAAT CAGATAAGGT
420

GGTAAACGGA TATATATTCT ATTATATTTA CGAATAAGTA TATTCCTATC CCATATATAA
480

CGTGTAAGAA GCTAAAGAAA TACGAATCCT TTGTTGTATA TGGAACCGAA ATAAAATCAA
540

TAATAAAATC TTCAAAGATT AGATATGCGA GTGTTATAAA AGTAACGGAG TATATCACAT
600

CTATCTGTTC GGAAGAAACT AGTTTATGGA ACAGCATCCC AATTGAGATA AAACATAAGA
660

TTATTAATAA TATAACAAT CATGATATGT ATATATTATA TAAAATAGA AAAAAAAAT
720

AA
722

(2) INFORMATION FOR SEQ ID NO:223:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 234 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:223:

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AAACAATGCG CTTTAATATC AAACATGCAG GTGGAATAGG ATTGTCGATA AGTAATATAC
60

GAGCTAAGGG TACTTATATA TCCGGTATAA ACGGCAAATC TATGGTATAG TACCTATGTT
120

AAGAATATAT AATAACACAG TTAGATATAT TAATCAGGGA GGTGATAAAA GACCAGGAGC
180

AATGTCGATT TATATAGAAC CATGGCAGCG TGATATATTC GATTTTCTAA GCTT
234

(2) INFORMATION FOR SEQ ID NO:224:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1025 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:224:

GGTTGCTCCT AACTTAATAA GATAATCCAC CAAGATAGTT TTATCCGTGG TAGATGCATA
60

CACAACAGGA GAATATCCTA ATTTATCTCT ATAGTTTATG GTTGTGATAT CTATAGTATA
120

TGGGACCGCC GAAAAACATG TATAATCGTC GTGACAATAG TTAAACATCG TGTTTAATAT
180

CGACATCATT TCATCATTTT TATTATATTC ATGTTTTATA TCGGAACAAA GCAAATTCAA
240

TATATTTAAA TTAGTGTTAT TGATGTGTCT AATTGTAAAT ATATGAATAG GATTCTTCAG
300

ACTATTATTT AGTTTACATA CATCAAATCC TTTTCTTATT AAAAACTCAA CAACTTTATA
360

ATCTATATTC TCATTACCAA GGTATTTATG CAATATGGTG TCTCCACATC TATGTACACT
420

GTTAATGTCA CCACCATGAT AAATAAGAAA CTTTATTACT TTAATTGTAA CATTTCGTATT
480

AAATGTAAAA TAACAATGAA ATGGTGTTTT ATCATATATA GATATCCCAT TTAAATTAGC
540

ACCTTTATTA AGCAGTAATA ATACAATTTT TTTCAACTCT TTTAATTTAA ATACGTGCAA
600

CGATGAACTT AAAAATGTAG CTAACATATC AGTGGCTATA TTATCATCCT GTTTTATATT
660

TGATATTATT CTTCTTATAT TATCCATTTT CTTCTTACAA ACTATTTAAA CGATAACCAA
720

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AATGTATTCA TGGGCTACTA ATAATAGCCA CATTACTAGA AAAAAAATTT TTTTCAATA
780

TTATGACATT ATTACTTAAG TATTATTGAT AAGTCCTTCA TTGTAAATG TAATAATATA
840

TATCGTTGTA TTTCTATAGG AATCCTCATC CAGTAACTAT GTTCTTGCA GTGCTTCATA
900

ATTACATAAA TCGCTTTATC AATGTTAGAA TAATACATAT ATGTATTTT GATAATATTT
960

TCTATATGTG ATCCATACAT TACTAAATTT TTAAATCTTA AAAAATTATC ATAATTGAGA
1020

AGCTT
1025

(2) INFORMATION FOR SEQ ID NO:225:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 305 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:225:

AAGCTTGGAT GAGCAATAAG AGTATACAAA ATTTAGTGTT TCAATTCGCT CATGGATCAG
60

AAGTAGAATA TATAGGTCAA TACGATATGA GATTTTAAA TAATATACCT ATTCATGATA
120

AGTTTGATGT GTTTTAAAT AAGCACATAC TATCGTATGT ACTTAGAGAT AAAATAAAGA
180

AATCAGACCA CAGATATGTA ATGTTTGGAT TTTGGTTATT TATCTCATTG GAAATGTGTT
240

ATATTCGATA AGGAACATCA TATGTCTGTT TCTATGATTC AGGAGGAATT ACCAAACGAA
300

TTCCA
305

(2) INFORMATION FOR SEQ ID NO:226:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1721 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: N

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(iv) ANTI-SENSE: N

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..1721

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:226:

ATG AAT TCG GAT CCG GCA ATA CTA TTA GTC TTG CTA TGT ACA TTT ACA
 48
 Met Asn Ser Asp Pro Ala Ile Leu Leu Val Leu Leu Cys Thr Phe Thr
 1 5 10 15

ACC GCA AAT GCA GAC ACA TTA TGT ATA GGT TAC CAT GCA AAT AAT TCA
 96
 Thr Ala Asn Ala Asp Thr Leu Cys Ile Gly Tyr His Ala Asn Asn Ser
 20 25 30

ACT GAC ACT GTT GAC ACA GTA CTA GAA AAG AAT GTA ACA GTA ACA CAC
 144
 Thr Asp Thr Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His
 35 40 45

TCT GTT AAC CTT CTA GAA GAC AGA CAT AAC GGG AAA CTA TGT AAA CTA
 192
 Ser Val Asn Leu Leu Glu Asp Arg His Asn Gly Lys Leu Cys Lys Leu
 50 55 60

AGA GGG GTA GCC CCA TTG CAT TTG GGT AAA TGT AAC ATT GCT GGA TGG
 240
 Arg Gly Val Ala Pro Leu His Leu Gly Lys Cys Asn Ile Ala Gly Trp
 65 70 75 80

CTC CTG GGA AAC CCA GAG TGT GAA TTA CTA TTC ACA GCA AGC TCA TGG
 288
 Leu Leu Gly Asn Pro Glu Cys Glu Leu Leu Phe Thr Ala Ser Ser Trp
 85 90 95

TCT TAC ATT GTG GAA ACA TCT AAT TCA GAC AAT GGG ACA TGT TAC CCA
 336
 Ser Tyr Ile Val Glu Thr Ser Asn Ser Asp Asn Gly Thr Cys Tyr Pro
 100 105 110

GGA GAT TTC ATC AAT TAT GAA GAG CTA AGA GAG CAG TTG AGC TCA GTG
 384
 Gly Asp Phe Ile Asn Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val
 115 120 125

TCA TCA TTT GAA AGA TTT GAG ATA TTC CCC AAG GCA AGT TCA TGG CCC
 432
 Ser Ser Phe Glu Arg Phe Glu Ile Phe Pro Lys Ala Ser Ser Trp Pro
 130 135 140

AAT CAT GAA ACG AAC ATA GGT GTG ACG GCA GCA TGT CCT TAT GCT GGA
 480
 Asn His Glu Thr Asn Ile Gly Val Thr Ala Ala Cys Pro Tyr Ala Gly
 145 150 155 160

GCA AAC AGC TTC TAC AGA AAC TTA ATA TGG CTG GTA AAA AAA GGA AAT
 528
 Ala Asn Ser Phe Tyr Arg Asn Leu Ile Trp Leu Val Lys Lys Gly Asn
 165 170 175

TCA TAC CCA AAG CTC AGC AAA TCC TAT ATT AAC AAT AAG GAG AAG GAA
 576

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Ser	Tyr	Pro	Lys	Leu	Ser	Lys	Ser	Tyr	Ile	Asn	Asn	Lys	Glu	Lys	Glu
			180					185					190		
GTC	CTC	GTG	CTA	TGG	GGC	ATT	CAC	CAT	CCA	CCT	ACC	AGT	ACT	GAC	CAA
624															
Val	Leu	Val	Leu	Trp	Gly	Ile	His	His	Pro	Pro	Thr	Ser	Thr	Asp	Gln
		195					200					205			
CAA	AGT	CTC	TAC	CAG	AAT	GCA	GAT	GCC	TAT	GTT	TTT	GTG	GGG	TCA	TCA
672															
Gln	Ser	Leu	Tyr	Gln	Asn	Ala	Asp	Ala	Tyr	Val	Phe	Val	Gly	Ser	Ser
	210					215					220				
AAA	TAC	AAC	AAG	AAA	TTC	AAG	CCA	GAA	ATA	GCA	ACA	AGA	CCC	AAG	GTG
720															
Lys	Tyr	Asn	Lys	Lys	Phe	Lys	Pro	Glu	Ile	Ala	Thr	Arg	Pro	Lys	Val
225					230					235					240
AGA	GGT	CAA	GCA	GGG	AGA	ATG	AAC	TAT	TAC	TGG	ACG	CTA	GTA	AAG	CCT
768															
Arg	Gly	Gln	Ala	Gly	Arg	Met	Asn	Tyr	Tyr	Trp	Thr	Leu	Val	Lys	Pro
				245					250					255	
GGA	GAC	ACA	ATA	ACA	TTC	GAA	GCA	ACT	GGA	AAT	CTA	GTG	GTA	CCA	AGA
816															
Gly	Asp	Thr	Ile	Thr	Phe	Glu	Ala	Thr	Gly	Asn	Leu	Val	Val	Pro	Arg
			260					265					270		
TAT	GCC	TTC	GCA	ATG	AAA	AGA	GGT	TCT	GGA	TCT	GGT	ATT	ATC	ATT	TCA
864															
Tyr	Ala	Phe	Ala	Met	Lys	Arg	Gly	Ser	Gly	Ser	Gly	Ile	Ile	Ile	Ser
	275						280					285			
GAT	ACA	CCA	GTC	CAC	GAT	TGT	AAT	ACG	ACT	TGT	CAA	ACA	CCC	AAA	GGT
912															
Asp	Thr	Pro	Val	His	Asp	Cys	Asn	Thr	Thr	Cys	Gln	Thr	Pro	Lys	Gly
	290					295					300				
GCT	ATA	AAC	ACC	AGC	CTT	CCA	TTT	CAG	AAT	ATA	CAT	CCA	GTC	ACA	ATT
960															
Ala	Ile	Asn	Thr	Ser	Leu	Pro	Phe	Gln	Asn	Ile	His	Pro	Val	Thr	Ile
305					310					315					320
GGA	GAA	TGT	CCA	AAA	TAT	GTC	AAA	AGC	ACA	AAA	TTG	AGA	ATG	GCT	ACA
1008															
Gly	Glu	Cys	Pro	Lys	Tyr	Val	Lys	Ser	Thr	Lys	Leu	Arg	Met	Ala	Thr
				325					330					335	
GGA	TTA	AGG	AAT	ATC	CCG	TCT	ATT	CAA	TCT	AGA	GGC	CTG	TTT	GGA	GCC
1056															
Gly	Leu	Arg	Asn	Ile	Pro	Ser	Ile	Gln	Ser	Arg	Gly	Leu	Phe	Gly	Ala
			340					345					350		
ATT	GCT	GGC	TTT	ATT	GAG	GGG	GGA	TGG	ACA	GGA	ATG	ATA	GAT	GGC	TGG
1104															
Ile	Ala	Gly	Phe	Ile	Glu	Gly	Gly	Trp	Thr	Gly	Met	Ile	Asp	Gly	Trp
		355					360					365			
TAC	GGT	TAT	CAC	CAT	CAG	AAT	GAG	CAG	GGA	TCA	GGA	TAT	GCA	GCC	GAC
1152															
Tyr	Gly	Tyr	His	His	Gln	Asn	Glu	Gln	Gly	Ser	Gly	Tyr	Ala	Ala	Asp
	370					375					380				
CGA	AAG	AGC	ACA	CAG	AAT	GCC	ATT	GAC	GGG	ATC	ACT	AAC	AAA	GTA	AAC
1200															
Arg	Lys	Ser	Thr	Gln	Asn	Ala	Ile	Asp	Gly	Ile	Thr	Asn	Lys	Val	Asn
385					390					395					400

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TCT GTT ATT GAA AAG ATG AAC ACA CAA TTC ACA GCA GTG GGT AAA GAA
 1248
 Ser Val Ile Glu Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu
 405 410 415

TTC AAC CAC CTG GAA AAA AGA ATA GAG AAT TTA AAC AAA AAG GTT GAT
 1296
 Phe Asn His Leu Glu Lys Arg Ile Glu Asn Leu Asn Lys Lys Val Asp
 420 425 430

GAT GGT TTT CTG GAT GTT TGG ACT TAC AAT GCC GAA CTG TTG GTT CTA
 1344
 Asp Gly Phe Leu Asp Val Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu
 435 440 445

TTG GAA AAT GAA AGA ACT TTG GAT TAT CAC GAT TCA AAT GTG AAG AAC
 1392
 Leu Glu Asn Glu Arg Thr Leu Asp Tyr His Asp Ser Asn Val Lys Asn
 450 455 460

CTA TAT GAG AAA GTA AGA AGC CAG CTA AAA AAC AAT GCC AAG GAA ATT
 1440
 Leu Tyr Glu Lys Val Arg Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile
 465 470 475 480

GGA AAT GGC TGC TTT GAA TTT TAC CAC AAA TGT GAT GAC ACG TGC ATG
 1488
 Gly Asn Gly Cys Phe Glu Phe Tyr His Lys Cys Asp Asp Thr Cys Met
 485 490 495

GAG AGC GTC AAA AAT GGG ACT TAT GAT TAC CCA AAA TAC TCA GAG GAA
 1536
 Glu Ser Val Lys Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu
 500 505 510

GCA AAA CTA AAC AGA GAG GAG ATA GAT GGG GTA AAG CTG GAA TCA ACA
 1584
 Ala Lys Leu Asn Arg Glu Glu Ile Asp Gly Val Lys Leu Glu Ser Thr
 515 520 525

AGG ATT TAC CAG ATT TTG GCG ATC TAT TCA ACT GTC GCC AGT TCA TTG
 1632
 Arg Ile Tyr Gln Ile Leu Ala Ile Tyr Ser Thr Val Ala Ser Ser Leu
 530 535 540

GTA CTG TTA GTC TCC CTG GGG GCA ATC AGT TTC TGG ATG TGC TCC AAT
 1680
 Val Leu Leu Val Ser Leu Gly Ala Ile Ser Phe Trp Met Cys Ser Asn
 545 550 555 560

GGG TCT TTA CAG TGC AGA ATA TGT ATT TAA AAT TAG GAT CC
 1721
 Gly Ser Leu Gln Cys Arg Ile Cys Ile . Asn . Asp
 565 570

(2) INFORMATION FOR SEQ ID NO:227:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 573 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:227:

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Met	Asn	Ser	Asp	Pro	Ala	Ile	Leu	Leu	Val	Leu	Leu	Cys	Thr	Phe	Thr
1				5					10					15	
Thr	Ala	Asn	Ala	Asp	Thr	Leu	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser
			20					25					30		
Thr	Asp	Thr	Val	Asp	Thr	Val	Leu	Glu	Lys	Asn	Val	Thr	Val	Thr	His
		35					40					45			
Ser	Val	Asn	Leu	Leu	Glu	Asp	Arg	His	Asn	Gly	Lys	Leu	Cys	Lys	Leu
	50					55					60				
Arg	Gly	Val	Ala	Pro	Leu	His	Leu	Gly	Lys	Cys	Asn	Ile	Ala	Gly	Trp
65					70					75					80
Leu	Leu	Gly	Asn	Pro	Glu	Cys	Glu	Leu	Leu	Phe	Thr	Ala	Ser	Ser	Trp
				85					90					95	
Ser	Tyr	Ile	Val	Glu	Thr	Ser	Asn	Ser	Asp	Asn	Gly	Thr	Cys	Tyr	Pro
			100					105					110		
Gly	Asp	Phe	Ile	Asn	Tyr	Glu	Glu	Leu	Arg	Glu	Gln	Leu	Ser	Ser	Val
		115					120					125			
Ser	Ser	Phe	Glu	Arg	Phe	Glu	Ile	Phe	Pro	Lys	Ala	Ser	Ser	Trp	Pro
		130				135					140				
Asn	His	Glu	Thr	Asn	Ile	Gly	Val	Thr	Ala	Ala	Cys	Pro	Tyr	Ala	Gly
145					150					155					160
Ala	Asn	Ser	Phe	Tyr	Arg	Asn	Leu	Ile	Trp	Leu	Val	Lys	Lys	Gly	Asn
				165					170					175	
Ser	Tyr	Pro	Lys	Leu	Ser	Lys	Ser	Tyr	Ile	Asn	Asn	Lys	Glu	Lys	Glu
			180					185					190		
Val	Leu	Val	Leu	Trp	Gly	Ile	His	His	Pro	Pro	Thr	Ser	Thr	Asp	Gln
		195					200					205			
Gln	Ser	Leu	Tyr	Gln	Asn	Ala	Asp	Ala	Tyr	Val	Phe	Val	Gly	Ser	Ser
	210					215					220				
Lys	Tyr	Asn	Lys	Lys	Phe	Lys	Pro	Glu	Ile	Ala	Thr	Arg	Pro	Lys	Val
225					230					235					240
Arg	Gly	Gln	Ala	Gly	Arg	Met	Asn	Tyr	Tyr	Trp	Thr	Leu	Val	Lys	Pro
				245					250					255	
Gly	Asp	Thr	Ile	Thr	Phe	Glu	Ala	Thr	Gly	Asn	Leu	Val	Val	Pro	Arg
			260					265					270		
Tyr	Ala	Phe	Ala	Met	Lys	Arg	Gly	Ser	Gly	Ser	Gly	Ile	Ile	Ile	Ser
		275					280					285			
Asp	Thr	Pro	Val	His	Asp	Cys	Asn	Thr	Thr	Cys	Gln	Thr	Pro	Lys	Gly
		290				295					300				
Ala	Ile	Asn	Thr	Ser	Leu	Pro	Phe	Gln	Asn	Ile	His	Pro	Val	Thr	Ile
305					310					315					320
Gly	Glu	Cys	Pro	Lys	Tyr	Val	Lys	Ser	Thr	Lys	Leu	Arg	Met	Ala	Thr
				325					330					335	
Gly	Leu	Arg	Asn	Ile	Pro	Ser	Ile	Gln	Ser	Arg	Gly	Leu	Phe	Gly	Ala
			340					345					350		
Ile	Ala	Gly	Phe	Ile	Glu	Gly	Gly	Trp	Thr	Gly	Met	Ile	Asp	Gly	Trp

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355		360		365											
Tyr	Gly	Tyr	His	His	Gln	Asn	Glu	Gln	Gly	Ser	Gly	Tyr	Ala	Ala	Asp
370						375					380				
Arg	Lys	Ser	Thr	Gln	Asn	Ala	Ile	Asp	Gly	Ile	Thr	Asn	Lys	Val	Asn
385					390					395					400
Ser	Val	Ile	Glu	Lys	Met	Asn	Thr	Gln	Phe	Thr	Ala	Val	Gly	Lys	Glu
				405					410					415	
Phe	Asn	His	Leu	Glu	Lys	Arg	Ile	Glu	Asn	Leu	Asn	Lys	Lys	Val	Asp
			420					425					430		
Asp	Gly	Phe	Leu	Asp	Val	Trp	Thr	Tyr	Asn	Ala	Glu	Leu	Leu	Val	Leu
		435					440					445			
Leu	Glu	Asn	Glu	Arg	Thr	Leu	Asp	Tyr	His	Asp	Ser	Asn	Val	Lys	Asn
450						455					460				
Leu	Tyr	Glu	Lys	Val	Arg	Ser	Gln	Leu	Lys	Asn	Asn	Ala	Lys	Glu	Ile
465					470					475					480
Gly	Asn	Gly	Cys	Phe	Glu	Phe	Tyr	His	Lys	Cys	Asp	Asp	Thr	Cys	Met
				485					490					495	
Glu	Ser	Val	Lys	Asn	Gly	Thr	Tyr	Asp	Tyr	Pro	Lys	Tyr	Ser	Glu	Glu
			500					505					510		
Ala	Lys	Leu	Asn	Arg	Glu	Glu	Ile	Asp	Gly	Val	Lys	Leu	Glu	Ser	Thr
		515					520					525			
Arg	Ile	Tyr	Gln	Ile	Leu	Ala	Ile	Tyr	Ser	Thr	Val	Ala	Ser	Ser	Leu
	530					535					540				
Val	Leu	Leu	Val	Ser	Leu	Gly	Ala	Ile	Ser	Phe	Trp	Met	Cys	Ser	Asn
545					550					555					560
Gly	Ser	Leu	Gln	Cys	Arg	Ile	Cys	Ile		Asn		Asp			
				565				570							

(2) INFORMATION FOR SEQ ID NO:228:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1414 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 1..1414
 - (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:228:

ATG	AAT	TCA	AAT	CAA	AAA	ATA	ATA	ACC	ATT	GGG	TCA	ATC	TGT	CTG	ATA
48															
Met	Asn	Ser	Asn	Gln	Lys	Ile	Ile	Thr	Ile	Gly	Ser	Ile	Cys	Leu	Ile
1				5				10					15		

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GTT GGA ATA GTT AGT CTA TTA TTG CAG ATA GGA AAT ATA GTC TCG TTA
96
Val Gly Ile Val Ser Leu Leu Leu Gln Ile Gly Asn Ile Val Ser Leu
20 25 30

TGG ATA AGC CAT TCA ATT CAG ACT GGA GAA AAA AAC CAC TCT GAG ATA
144
Trp Ile Ser His Ser Ile Gln Thr Gly Glu Lys Asn His Ser Glu Ile
35 40 45

TGC AAC CAA AAT ATC ATT ACA TAT GAA AAC AAC ACA TGG GTG AAC CAA
192
Cys Asn Gln Asn Ile Ile Thr Tyr Glu Asn Asn Thr Trp Val Asn Gln
50 55 60

ACT TAT GTA AAC ATT AGC AAT ACC AAC ATT GCT GAT GGA CAG GGC GTG
240
Thr Tyr Val Asn Ile Ser Asn Thr Asn Ile Ala Asp Gly Gln Gly Val
65 70 75 80

ACT TCA ATA ATA CTA GCC GGC AAT CCC CCT CTT TGC CCA ATA ATT GGG
288
Thr Ser Ile Ile Leu Ala Gly Asn Pro Pro Leu Cys Pro Ile Ile Gly
85 90 95

TGG GCT ATA TAC AGC AAA AAC AAT AGC ATA AGG ATT GGT CCC AAA GGA
336
Trp Ala Ile Tyr Ser Lys Asn Asn Ser Ile Arg Ile Gly Pro Lys Gly
100 105 110

AAC ATT TTT GTC ATA AAA AAA CCA TCC ATT TCA TGC TCT CAC TTG GAG
384
Asn Ile Phe Val Ile Lys Lys Pro Ser Ile Ser Cys Ser His Leu Glu
115 120 125

TGC AAA ACC TTT TTC CTG ACC CAA GGT GCT TTG CTA AAT GAC AGG CAT
432
Cys Lys Thr Phe Phe Leu Thr Gln Gly Ala Leu Leu Asn Asp Arg His
130 135 140

CCT AAT GGA ACC GTC AAG GAC AGG AGC CCT TAC CGA ACC TTA ATG AGC
480
Pro Asn Gly Thr Val Lys Asp Arg Ser Pro Tyr Arg Thr Leu Met Ser
145 150 155 160

TGC CCG ATC GGT GAA GCT CCA TCT CCG TAT AAT TCA AGA TTC GAA TCA
528
Cys Pro Ile Gly Glu Ala Pro Ser Pro Tyr Asn Ser Arg Phe Glu Ser
165 170 175

GTT GCT TGG TCA GCA AGT GCA TGC CAT GAT GGA ATG GGA TGG CTA ACA
576
Val Ala Trp Ser Ala Ser Ala Cys His Asp Gly Met Gly Trp Leu Thr
180 185 190

ATC GGG ATT TCC GGT CCA GAT AAT GGA GCA GTG GCT GTT TTG AAA TAC
624
Ile Gly Ile Ser Gly Pro Asp Asn Gly Ala Val Ala Val Leu Lys Tyr
195 200 205

AAT GGT ATA ATA ACA GAT ACA ATA AAA AGT TGG AGA AAC AAA ATA CTA
672
Asn Gly Ile Ile Thr Asp Thr Ile Lys Ser Trp Arg Asn Lys Ile Leu
210 215 220

AGA ACA CAA GAG TCA GAA TGT GTT TGT ATA AAC GGT TCA TGT TTT ACT
720

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Arg 225	Thr	Gln	Glu	Ser	Glu 230	Cys	Val	Cys	Ile	Asn 235	Gly	Ser	Cys	Phe	Thr 240
ATA 768	ATG	ACT	GAT	GGC	CCA	AGC	AAT	GGG	CAA	GCC	TCG	TAC	AAA	ATA	TTC
Ile	Met	Thr	Asp	Gly 245	Pro	Ser	Asn	Gly	Gln 250	Ala	Ser	Tyr	Lys	Ile 255	Phe
AAA 816	ATG	GAG	AAA	GGG	AAG	ATT	ATT	AAG	TCA	GTT	GAG	CTG	GAT	GCA	CCT
Lys	Met	Glu	Lys	Gly 260	Lys	Ile	Ile	Lys	Ser 265	Val	Glu	Leu	Asp 270	Ala	Pro
AAT 864	TAC	CAC	TAT	GAG	GAA	TGC	TCC	TGT	TAC	CCT	GAT	ACA	GGC	AAA	GTG
Asn	Tyr	His 275	Tyr	Glu	Glu	Cys	Ser 280	Cys	Tyr	Pro	Asp	Thr 285	Gly	Lys	Val
GTG 912	TGT	GTG	TGC	AGA	GAC	AAT	TGG	CAT	GCT	TCA	AAT	CGA	CCG	TGG	GTC
Val	Cys 290	Val	Cys	Arg	Asp	Asn 295	Trp	His	Ala	Ser	Asn 300	Arg	Pro	Trp	Val
TCT 960	TTC	GAT	CAG	AAT	CTT	GAT	TAT	CAG	ATA	GGG	TAC	ATA	TGC	AGT	GGG
Ser 305	Phe	Asp	Gln	Asn 310	Leu	Asp	Tyr	Gln	Ile	Gly 315	Tyr	Ile	Cys	Ser	Gly 320
GTT 1008	TTC	GGT	GAT	AAT	CCG	CGT	TCT	AAT	GAT	GGG	AAA	GGC	AAT	TGT	GGC
Val	Phe	Gly	Asp	Asn 325	Pro	Arg	Ser	Asn 330	Asp	Gly	Lys	Gly	Asn 335	Cys	Gly
CCA 1056	GTA	CTT	TCT	AAT	GGA	GCA	AAT	GGG	GTG	AAA	GGA	TTC	TCA	TTT	AGA
Pro	Val	Leu	Ser 340	Asn	Gly	Ala	Asn 345	Gly	Val	Lys	Gly	Phe	Ser 350	Phe	Arg
TAT 1104	GGC	AAT	GGT	GTT	TGG	ATA	GGA	AGA	ACT	AAA	AGT	ATC	AGC	TCT	AGA
Tyr	Gly	Asn 355	Gly	Val	Trp	Ile	Gly 360	Arg	Thr	Lys	Ser	Ile 365	Ser	Ser	Arg
AGT 1152	GGA	TTT	GAG	ATG	ATT	TGG	GAT	CCA	AAT	GGA	TGG	ACG	GAA	ACT	GAT
Ser	Gly 370	Phe	Glu	Met	Ile	Trp 375	Asp	Pro	Asn	Gly	Trp 380	Thr	Glu	Thr	Asp
AGT 1200	AGT	TTC	TCT	ATA	AAG	CAG	GAT	ATT	ATA	GCA	TTA	ACT	GAT	TGG	TCA
Ser 385	Ser	Phe	Ser	Ile	Lys 390	Gln	Asp	Ile	Ile	Ala 395	Leu	Thr	Asp	Trp	Ser 400
GGA 1248	TAC	AGT	GGA	AGT	TTT	GTC	CAA	CAT	CCT	GAA	TTA	ACA	GGA	ATG	AAC
Gly	Tyr	Ser	Gly	Ser 405	Phe	Val	Gln	His 410	Pro	Glu	Leu	Thr	Gly	Met 415	Asn
TGC 1296	ATA	AGG	CCT	TGT	TTT	TGG	GTA	GAG	TTA	ATC	AGA	GGA	CAA	CCC	AAG
Cys	Ile	Arg	Pro 420	Cys	Phe	Trp	Val	Glu 425	Leu	Ile	Arg	Gly	Gln 430	Pro	Lys
GAG 1344	AGC	ACA	ATC	TGG	ACT	AGT	GGA	AGC	AGC	ATT	TCT	TTC	TGT	GGC	GTG
Glu	Ser	Thr 435	Ile	Trp	Thr	Ser	Gly 440	Ser	Ser	Ile	Ser	Phe 445	Cys	Gly	Val

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GAC AAT GAA ACC GCA AGC TGG TCA TGG CCA GAC GGA GCT GAT CTG CCA
1392

Asp Asn Glu Thr Ala Ser Trp Ser Trp Pro Asp Gly Ala Asp Leu Pro
450 455 460

TTC ACC ATT GAC AAG TAG ATC T

1414

Phe Thr Ile Asp Lys . Ile
465 470

(2) INFORMATION FOR SEQ ID NO:229:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 471 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:229:

Met Asn Ser Asn Gln Lys Ile Ile Thr Ile Gly Ser Ile Cys Leu Ile
1 5 10 15

Val Gly Ile Val Ser Leu Leu Leu Gln Ile Gly Asn Ile Val Ser Leu
20 25 30

Trp Ile Ser His Ser Ile Gln Thr Gly Glu Lys Asn His Ser Glu Ile
35 40 45

Cys Asn Gln Asn Ile Ile Thr Tyr Glu Asn Asn Thr Trp Val Asn Gln
50 55 60

Thr Tyr Val Asn Ile Ser Asn Thr Asn Ile Ala Asp Gly Gln Gly Val
65 70 75 80

Thr Ser Ile Ile Leu Ala Gly Asn Pro Pro Leu Cys Pro Ile Ile Gly
85 90 95

Trp Ala Ile Tyr Ser Lys Asn Asn Ser Ile Arg Ile Gly Pro Lys Gly
100 105 110

Asn Ile Phe Val Ile Lys Lys Pro Ser Ile Ser Cys Ser His Leu Glu
115 120 125

Cys Lys Thr Phe Phe Leu Thr Gln Gly Ala Leu Leu Asn Asp Arg His
130 135 140

Pro Asn Gly Thr Val Lys Asp Arg Ser Pro Tyr Arg Thr Leu Met Ser
145 150 155 160

Cys Pro Ile Gly Glu Ala Pro Ser Pro Tyr Asn Ser Arg Phe Glu Ser
165 170 175

Val Ala Trp Ser Ala Ser Ala Cys His Asp Gly Met Gly Trp Leu Thr
180 185 190

Ile Gly Ile Ser Gly Pro Asp Asn Gly Ala Val Ala Val Leu Lys Tyr
195 200 205

Asn Gly Ile Ile Thr Asp Thr Ile Lys Ser Trp Arg Asn Lys Ile Leu
210 215 220

Arg Thr Gln Glu Ser Glu Cys Val Cys Ile Asn Gly Ser Cys Phe Thr
225 230 235 240

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Ile	Met	Thr	Asp	Gly 245	Pro	Ser	Asn	Gly	Gln 250	Ala	Ser	Tyr	Lys	Ile 255	Phe
Lys	Met	Glu	Lys 260	Gly	Lys	Ile	Ile	Lys 265	Ser	Val	Glu	Leu	Asp 270	Ala	Pro
Asn	Tyr	His 275	Tyr	Glu	Glu	Cys	Ser 280	Cys	Tyr	Pro	Asp	Thr 285	Gly	Lys	Val
Val	Cys 290	Val	Cys	Arg	Asp	Asn 295	Trp	His	Ala	Ser	Asn 300	Arg	Pro	Trp	Val
Ser 305	Phe	Asp	Gln	Asn 310	Leu	Asp	Tyr	Gln	Ile	Gly 315	Tyr	Ile	Cys	Ser	Gly 320
Val	Phe	Gly	Asp	Asn 325	Pro	Arg	Ser	Asn	Asp 330	Gly	Lys	Gly	Asn	Cys 335	Gly
Pro	Val	Leu	Ser 340	Asn	Gly	Ala	Asn	Gly 345	Val	Lys	Gly	Phe	Ser 350	Phe	Arg
Tyr	Gly 355	Asn	Gly	Val	Trp	Ile	Gly 360	Arg	Thr	Lys	Ser	Ile 365	Ser	Ser	Arg
Ser 370	Gly	Phe	Glu	Met	Ile	Trp 375	Asp	Pro	Asn	Gly	Trp 380	Thr	Glu	Thr	Asp
Ser 385	Ser	Phe	Ser	Ile	Lys 390	Gln	Asp	Ile	Ile	Ala 395	Leu	Thr	Asp	Trp	Ser 400
Gly	Tyr	Ser	Gly	Ser 405	Phe	Val	Gln	His	Pro 410	Glu	Leu	Thr	Gly	Met 415	Asn
Cys	Ile	Arg	Pro 420	Cys	Phe	Trp	Val	Glu 425	Leu	Ile	Arg	Gly	Gln 430	Pro	Lys
Glu	Ser	Thr 435	Ile	Trp	Thr	Ser	Gly 440	Ser	Ser	Ile	Ser	Phe 445	Cys	Gly	Val
Asp	Asn 450	Glu	Thr	Ala	Ser	Trp 455	Ser	Trp	Pro	Asp	Gly 460	Ala	Asp	Leu	Pro
Phe 465	Thr	Ile	Asp	Lys	.	Ile 470									

(A) LENGTH: 1501 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(A) NAME/KEY: CDS
(B) LOCATION: 1..1501
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:230:

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ATG AAT TCT CAA GGC ACC AAA CGA TCA TAT GAA CAA ATG GAG ACT GGT
 48
 Met Asn Ser Gln Gly Thr Lys Arg Ser Tyr Glu Gln Met Glu Thr Gly
 1 5 10 15

GGG GAA CGC CAG GAT GCC ACA GAA ATC AGA GCA TCT GTC GGA AGA ATG
 96
 Gly Glu Arg Gln Asp Ala Thr Glu Ile Arg Ala Ser Val Gly Arg Met
 20 25 30

ATT GGT GGA ATC GGA AGA TTC TAC ATC CAA ATG TGC ACT GAA CTC AAA
 144
 Ile Gly Gly Ile Gly Arg Phe Tyr Ile Gln Met Cys Thr Glu Leu Lys
 35 40 45

CTC AGT GAC TAT GAG GGA CGA CTA ATT CAA AAT AGC ATA ACA ATA GAG
 192
 Leu Ser Asp Tyr Glu Gly Arg Leu Ile Gln Asn Ser Ile Thr Ile Glu
 50 55 60

AGA ATG GTG CTC TCT GCT TTT GAT GAG AGA AGG AAT AAA TAC CTA GAA
 240
 Arg Met Val Leu Ser Ala Phe Asp Glu Arg Arg Asn Lys Tyr Leu Glu
 65 70 75 80

GAG CAT CCC AGT GCT GGG AAG GAT CCT AAG AAA ACT GGA GGA CCC ATA
 288
 Glu His Pro Ser Ala Gly Lys Asp Pro Lys Lys Thr Gly Gly Pro Ile
 85 90 95

TAT AGA AGG GTA GAC GGA AAA TGG ATG AGA GAA CTC ATC CTT TAT GAC
 336
 Tyr Arg Arg Val Asp Gly Lys Trp Met Arg Glu Leu Ile Leu Tyr Asp
 100 105 110

AAA GAA GAA ATA AGG AGA GTT TGG CGC CAA GCA AAC AAT GGT GAG GAT
 384
 Lys Glu Glu Ile Arg Arg Val Trp Arg Gln Ala Asn Asn Gly Glu Asp
 115 120 125

GCA ACA GCC GGT CTT ACT CAC ATC ATG ATT TGG CAC TCC AAT CTT AAT
 432
 Ala Thr Ala Gly Leu Thr His Ile Met Ile Trp His Ser Asn Leu Asn
 130 135 140

GAT GCC ACC TAT CAG AGA ACA AGA GCG CTT GTT CGC ACT GGA ATG GAT
 480
 Asp Ala Thr Tyr Gln Arg Thr Arg Ala Leu Val Arg Thr Gly Met Asp
 145 150 155 160

CCC AGA ATG TGC TCC CTA ATG CAA GGT TCA ACA CTT CCC AGA AGG TCT
 528
 Pro Arg Met Cys Ser Leu Met Gln Gly Ser Thr Leu Pro Arg Arg Ser
 165 170 175

GGG GCC GCA GGT GCT GCA GTG AAA GGA GTT GGA ACA ATA GCA ATG GAG
 576
 Gly Ala Ala Gly Ala Ala Val Lys Gly Val Gly Thr Ile Ala Met Glu
 180 185 190

TTA ATC AGA ATG ATC AAA CGT GGA ATC AAT GAC CGA AAC TTC TGG AGG
 624
 Leu Ile Arg Met Ile Lys Arg Gly Ile Asn Asp Arg Asn Phe Trp Arg
 195 200 205

GGT GAA AAT GGA CGA AGG ACA AGG ATT GCA TAT GAA AGA ATG TGC AAT
 672

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Gly 210	Glu	Asn	Gly	Arg	Arg	Thr 215	Arg	Ile	Ala	Tyr	Glu 220	Arg	Met	Cys	Asn
ATT 720	CTC	AAA	GGA	AAA	TTT	CAG	ACA	GCT	GCC	CAG	AGG	GCA	ATG	ATG	GAT
Ile 225	Leu	Lys	Gly	Lys	Phe 230	Gln	Thr	Ala	Ala	Gln 235	Arg	Ala	Met	Met	Asp 240
CAA 768	GTA	AGA	GAA	AGT	CGA	AAC	CCA	GGA	AAC	GCT	GAA	ATT	GAA	GAT	CTC
Gln	Val	Arg	Glu	Ser 245	Arg	Asn	Pro	Gly	Asn 250	Ala	Glu	Ile	Glu	Asp 255	Leu
ATT 816	TTC	CTG	GCA	CGG	TCA	GCA	CTT	ATT	CTA	AGG	GGG	TCA	GTT	GCA	CAT
Ile	Phe	Leu	Ala 260	Arg	Ser	Ala	Leu	Ile 265	Leu	Arg	Gly	Ser	Val 270	Ala	His
AAG 864	TCC	TGC	CTG	CCT	GCT	TGT	GTG	TAT	GGG	CTT	GCA	GTA	GCA	AGT	GGG
Lys	Ser	Cys 275	Leu	Pro	Ala	Cys	Val 280	Tyr	Gly	Leu	Ala	Val 285	Ala	Ser	Gly
CAT 912	GAC	TTT	GAA	AGA	GAA	GGA	TAT	TCA	CTG	GTC	GGG	ATA	GAC	CCC	TTC
His	Asp 290	Phe	Glu	Arg	Glu	Gly 295	Tyr	Ser	Leu	Val	Gly 300	Ile	Asp	Pro	Phe
AAA 960	TTA	CTT	CAA	AAC	AGT	CAA	GTG	TTC	AGC	CTG	ATC	AGA	CCA	AAT	GAA
Lys 305	Leu	Leu	Gln	Asn 310	Ser	Gln	Val	Phe	Ser	Leu 315	Ile	Arg	Pro	Asn	Glu 320
AAC 1008	CCA	GCT	CAC	AAG	AGT	CAA	TTG	GTG	TGG	ATG	GCA	TGC	CAT	TCT	GCT
Asn	Pro	Ala	His	Lys 325	Ser	Gln	Leu	Val	Trp 330	Met	Ala	Cys	His	Ser 335	Ala
GCA 1056	TTT	GAG	GAT	TTA	AGA	ATA	TCA	AGT	TTC	ATA	AGA	GGG	AAG	AAA	GTG
Ala	Phe	Glu	Asp 340	Leu	Arg	Ile	Ser	Ser 345	Phe	Ile	Arg	Gly	Lys 350	Lys	Val
GTT 1104	CCA	AGA	GGA	AAG	CTT	TCC	ACA	AGA	GGG	GTT	CAG	ATT	GCT	TCA	AAT
Val	Pro	Arg 355	Gly	Lys	Leu	Ser	Thr 360	Arg	Gly	Val	Gln 365	Ile	Ala	Ser	Asn
GAG 1152	AAT	GTG	GAA	GCT	ATG	GAC	TCT	AGT	ACC	CTA	AAA	CTA	AGA	AGC	AGA
Glu	Asn 370	Val	Glu	Ala	Met	Asp 375	Ser	Ser	Thr	Leu	Lys 380	Leu	Arg	Ser	Arg
TAT 1200	TGG	GCC	ATA	AGG	ACC	AGA	AGT	GGA	GGA	AAT	ACC	AAC	CAA	CAG	AAG
Tyr 385	Trp	Ala	Ile	Arg	Thr 390	Arg	Ser	Gly	Gly	Asn 395	Thr	Asn	Gln	Gln	Lys 400
GCA 1248	TCT	GCG	GGC	CAG	ATC	AGT	GTG	CAA	CCT	ACA	TTC	TCA	GTG	CAA	CGG
Ala	Ser	Ala	Gly	Gln 405	Ile	Ser	Val	Gln	Pro 410	Thr	Phe	Ser	Val	Gln 415	Arg
AAT 1296	CTC	CCT	TTT	GAA	AGA	GCA	ACC	GTT	ATG	GCA	GCT	TTC	AGC	GGG	AAT
Asn	Leu	Pro	Phe 420	Glu	Arg	Ala	Thr	Val 425	Met	Ala	Ala	Phe	Ser	Gly	Asn 430

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AAT GAG GGA CGG ACA TCA GAC ATG CGA ACG GAA GTT ATA AGG ATG ATG
1344
Asn Glu Gly Arg Thr Ser Asp Met Arg Thr Glu Val Ile Arg Met Met
435 440 445

GAA AGT GCA AAG CCA GAA GAT TTG TCC TTC CAG GGG CGG GGA GTC TTC
1392
Glu Ser Ala Lys Pro Glu Asp Leu Ser Phe Gln Gly Arg Gly Val Phe
450 455 460

GAG CTC TCG GAC GAA AAG GCA ACG AAC CCG ATC GTG CCT TCC TTT GAC
1440
Glu Leu Ser Asp Glu Lys Ala Thr Asn Pro Ile Val Pro Ser Phe Asp
465 470 475 480

ATG AGT AAT GAA GGG TCT TAT TTC TTC GGA GAC AAT GCA GAG GAG TAT
1488
Met Ser Asn Glu Gly Ser Tyr Phe Phe Gly Asp Asn Ala Glu Glu Tyr
485 490 495

GAC AAT TGA ATT C
1501
Asp Asn . Ile
500

(2) INFORMATION FOR SEQ ID NO:231:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 500 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:231:

Met Asn Ser Gln Gly Thr Lys Arg Ser Tyr Glu Gln Met Glu Thr Gly
1 5 10 15

Gly Glu Arg Gln Asp Ala Thr Glu Ile Arg Ala Ser Val Gly Arg Met
20 25 30

Ile Gly Gly Ile Gly Arg Phe Tyr Ile Gln Met Cys Thr Glu Leu Lys
35 40 45

Leu Ser Asp Tyr Glu Gly Arg Leu Ile Gln Asn Ser Ile Thr Ile Glu
50 55 60

Arg Met Val Leu Ser Ala Phe Asp Glu Arg Arg Asn Lys Tyr Leu Glu
65 70 75 80

Glu His Pro Ser Ala Gly Lys Asp Pro Lys Lys Thr Gly Gly Pro Ile
85 90 95

Tyr Arg Arg Val Asp Gly Lys Trp Met Arg Glu Leu Ile Leu Tyr Asp
100 105 110

Lys Glu Glu Ile Arg Arg Val Trp Arg Gln Ala Asn Asn Gly Glu Asp
115 120 125

Ala Thr Ala Gly Leu Thr His Ile Met Ile Trp His Ser Asn Leu Asn
130 135 140

Asp Ala Thr Tyr Gln Arg Thr Arg Ala Leu Val Arg Thr Gly Met Asp
145 150 155 160

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Pro Arg Met Cys Ser Leu Met Gln Gly Ser Thr Leu Pro Arg Arg Ser
165 170 175

Gly Ala Ala Gly Ala Ala Val Lys Gly Val Gly Thr Ile Ala Met Glu
180 185 190

Leu Ile Arg Met Ile Lys Arg Gly Ile Asn Asp Arg Asn Phe Trp Arg
195 200 205

Gly Glu Asn Gly Arg Arg Thr Arg Ile Ala Tyr Glu Arg Met Cys Asn
210 215 220

Ile Leu Lys Gly Lys Phe Gln Thr Ala Ala Gln Arg Ala Met Met Asp
225 230 235 240

Gln Val Arg Glu Ser Arg Asn Pro Gly Asn Ala Glu Ile Glu Asp Leu
245 250 255

Ile Phe Leu Ala Arg Ser Ala Leu Ile Leu Arg Gly Ser Val Ala His
260 265 270

Lys Ser Cys Leu Pro Ala Cys Val Tyr Gly Leu Ala Val Ala Ser Gly
275 280 285

His Asp Phe Glu Arg Glu Gly Tyr Ser Leu Val Gly Ile Asp Pro Phe
290 295 300

Lys Leu Leu Gln Asn Ser Gln Val Phe Ser Leu Ile Arg Pro Asn Glu
305 310 315 320

Asn Pro Ala His Lys Ser Gln Leu Val Trp Met Ala Cys His Ser Ala
325 330 335

Ala Phe Glu Asp Leu Arg Ile Ser Ser Phe Ile Arg Gly Lys Lys Val
340 345 350

Val Pro Arg Gly Lys Leu Ser Thr Arg Gly Val Gln Ile Ala Ser Asn
355 360 365

Glu Asn Val Glu Ala Met Asp Ser Ser Thr Leu Lys Leu Arg Ser Arg
370 375 380

Tyr Trp Ala Ile Arg Thr Arg Ser Gly Gly Asn Thr Asn Gln Gln Lys
385 390 395 400

Ala Ser Ala Gly Gln Ile Ser Val Gln Pro Thr Phe Ser Val Gln Arg
405 410 415

Asn Leu Pro Phe Glu Arg Ala Thr Val Met Ala Ala Phe Ser Gly Asn
420 425 430

Asn Glu Gly Arg Thr Ser Asp Met Arg Thr Glu Val Ile Arg Met Met
435 440 445

Glu Ser Ala Lys Pro Glu Asp Leu Ser Phe Gln Gly Arg Gly Val Phe
450 455 460

Glu Leu Ser Asp Glu Lys Ala Thr Asn Pro Ile Val Pro Ser Phe Asp
465 470 475 480

Met Ser Asn Glu Gly Ser Tyr Phe Phe Gly Asp Asn Ala Glu Glu Tyr
485 490 495

Asp Asn . Ile
500

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What is claimed is:

1. A recombinant swinepox virus comprising a foreign DNA inserted into a swinepox virus genome, wherein the foreign DNA is inserted into an EcoRI site within a region corresponding to a 3.2 Kb subfragment of the HindIII K fragment which contains both a HindIII and an EcoRI site, of the swinepox virus genome and is capable of being expressed in a host cell into which the virus is introduced.
2. A recombinant swinepox virus comprising a foreign DNA inserted into a swinepox virus genome, wherein the foreign DNA is inserted into a) an AccI site within a region corresponding to a 3.6 Kb HindIII to BglII subfragment of the HindIII M fragment and b) an EcoRI site within a region corresponding to a 3.2 Kb subfragment of the HindIII K fragment which contains both a HindIII and EcoRI site, of the swinepox virus genome and is capable of being expressed in a host cell into which the virus is introduced.
3. The recombinant swinepox virus of claim 1, wherein the foreign DNA encodes a polypeptide.
4. The recombinant swinepox virus of claim 1, wherein the foreign DNA encodes E. coli beta-galactosidase or beta-glucuronidase.

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5. The recombinant swinepox virus of claim 1, wherein the foreign DNA is under control of a heterologous upstream promoter.
6. The recombinant swinepox virus of claim 5, wherein the promoter is: synthetic pox viral promoter, pox synthetic late promoter 1, pox early promoter 2, pox synthetic late promoter 2 early promoter 2, pox synthetic early promoter 2, pox O1L promoter, pox I4L promoter, pox I3L promoter, pox I2L promoter, pox I1L promoter, pox E10R promoter, PRV gX, HSV-1 alpha 4, internal ribosomal entry site, and HCMV immediate early.
7. The recombinant swinepox virus of claim 1, wherein the polypeptide is: swine influenza virus hemagglutinin, swine influenza virus neurominidase, swine influenza virus matrix and swine influenza virus nucleoprotein.
8. The recombinant swinepox virus of claim 7, which is designated S-SPV-120.
9. The recombinant swinepox virus of claim 7, which is designated S-SPV-121.
10. The recombinant swinepox virus of claim 7, which is designated S-SPV-122.
11. The recombinant swinepox virus of claim 1, wherein the foreign DNA encodes a cytokine.
12. The recombinant swinepox virus of claim 1, wherein the polypeptide is: swine influenza

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virus hemagglutinin, swine influenza virus neurominidase, swine influenza virus matrix, swine influenza virus nucleoprotein, pseudorabies virus virus glycoprotein B, pseudorabies virus glycoprotein C, pseudorabies virus glycoprotein D, porcine respiratory and reproductive virus ORF2, porcine respiratory and reproductive virus ORF3, porcine respiratory and reproductive virus ORF4, porcine respiratory and reproductive virus ORF5, porcine respiratory, and reproductive virus ORF6, and porcine respiratory and reproductive virus ORF7.

13. The recombinant swinepox virus of claim 12, which is designated S-SPV-131.
14. The recombinant swinepox virus of claim 12, which is designated S-SPV-132.
15. The recombinant swinepox virus of claim 1, wherein the polypeptide is: feline leukemia virus surface protein, feline leukemia virus transmembrane protein, feline leukemia virus gag, feline leukemia virus transmembrane protease, feline immunodeficiency virus gag/protease, feline immunodeficiency virus envelope, feline leukemia virus gag/protease, feline leukemia virus envelope, canine parvovirus VP2, and canine parvovirus VP1/2.
16. The recombinant swinepox virus of claim 15, which is designated S-SPV-127.

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17. The recombinant swinepox virus of claim 15, which is designated S-SPV-128.
18. The recombinant swinepox virus of claim 15, which is designated S-SPV-205.
19. The recombinant swinepox virus of claim 15, which is designated S-SPV-206.
20. The recombinant swinepox virus of claim 15, which is designated S-SPV-207.
21. The recombinant swinepox virus of claim 1, wherein the polypeptide is: bovine cytokine interleukin-12 protein 35, bovine cytokine interleukin-12 protein 40, Bovine Respiratory Syncytial Virus glycoprotein G, Newcastle Disease fusion, Infectious Rhinotracheitis Virus glycoprotein D, Canine Distemper Virus fusion, Canine Distemper Virus Hemagglutinin, DV HA, Bovine Viral Diarrhea Virus type 1 glycoprotein 45, Bovine Viral Diarrhea Virus type 1 glycoprotein 48, Bovine Viral Diarrhea Virus type 1 glycoprotein 53, Bovine Viral Diarrhea Virus type 2 glycoprotein 53.
22. The recombinant swinepox virus of claim 21, which is designated S-SPV-142.
23. The recombinant swinepox virus of claim 21, which is designated S-SPV-143.
24. The recombinant swinepox virus of claim 21, which is designated S-SPV-176.

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25. The recombinant swinepox virus of claim 21, which is designated S-SPV-184.
26. The recombinant swinepox virus of claim 21, which is designated S-SPV-185.
27. The recombinant swinepox virus of claim 21, which is designated S-SPV-188.
28. The recombinant swinepox virus of claim 21, which is designated S-SPV-202.
29. A recombinant swinepox virus designated S-SPV-164.
30. A recombinant swinepox virus designated S-SPV-183.
31. A recombinant swinepox virus designated S-SPV-198.
32. A recombinant swinepox virus designated S-SPV-200.
33. A recombinant swinepox virus designated S-SPV-217.
34. A vaccine useful for immunizing an animal against swinepox virus which comprises an effective immunizing amount of the recombinant swinepox virus of claims 1 and a suitable carrier.
35. A method of immunizing an animal against a pathogen which comprises administering to the

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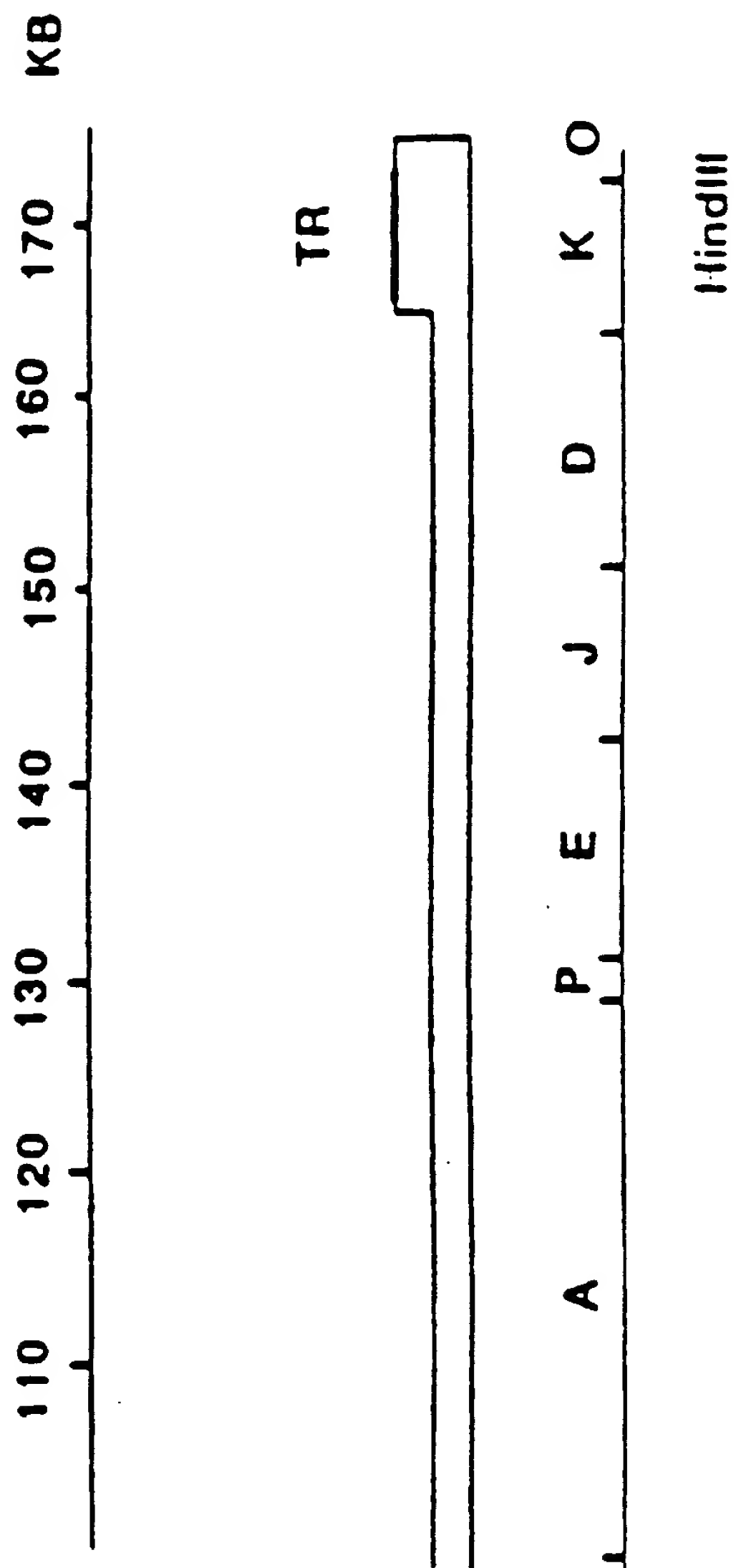
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animal an effective immunizing dose of the
vaccine of claim 34.

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FIGURE 1B



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FIGURE 2A

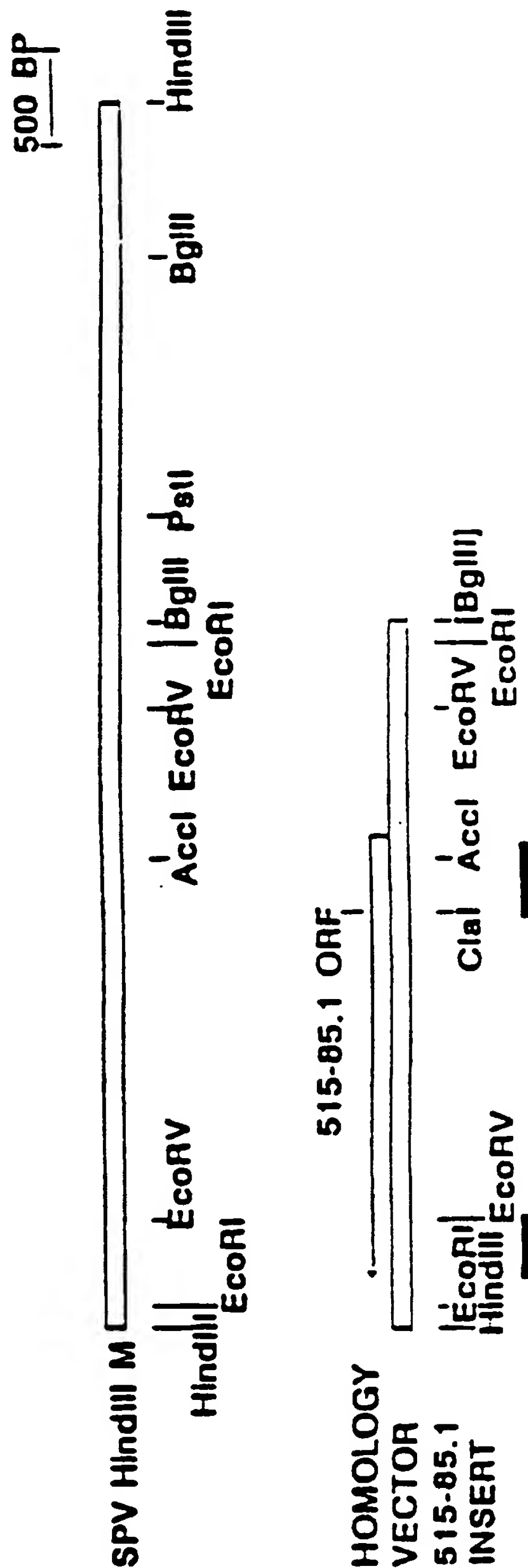


FIGURE 2B

```
(A) VV MFMYPEFARKALSKLISKKLNIEKVSSKHQLVLLDYGLHGLLPKSLYLEAINSDILNVRFFPPEIINVT  
orf 01L   :: :: :: :: :: :: :: :: :: :: :: :: :: :: :: :: :: :: :: :: :: :: ::  
(B) SPV MPSYMPKNARKVISKIISLOLDIKKLPPKYINTMLEFGLHG NLPACMYKDASVDINNIRFLPYNCVMVK  
AccI-Clal      |
```

ACCI

	80	90	100	110	120
(A) VV	DIVKALON	SCRVDEYL	KAVSLYHKNSL	MVSGPNVVK-L	LMIEYNLLTHSDLEWL
orf 01L	:	:	:	:	:
(B) SPV	DLINVIKSS	VIDTLHQSVL	KHRRALIDYGD	ODIITLMI	INKLLSIDDISYILDKKI
AccI-Clai					

Claim

FIGURE 2C

(C) W OH O1L VLNDQYAKIVIFFNTIIEYIATIYYALTVLNNTYTNVKHFVSKVLHTVMEA 570 580 590 600 610

(D) SPV Ecorv·EcorI SLNEYYSKIVILINVILEYMSIILYAILVKRFNNIKEFISKVVNTVLÉS

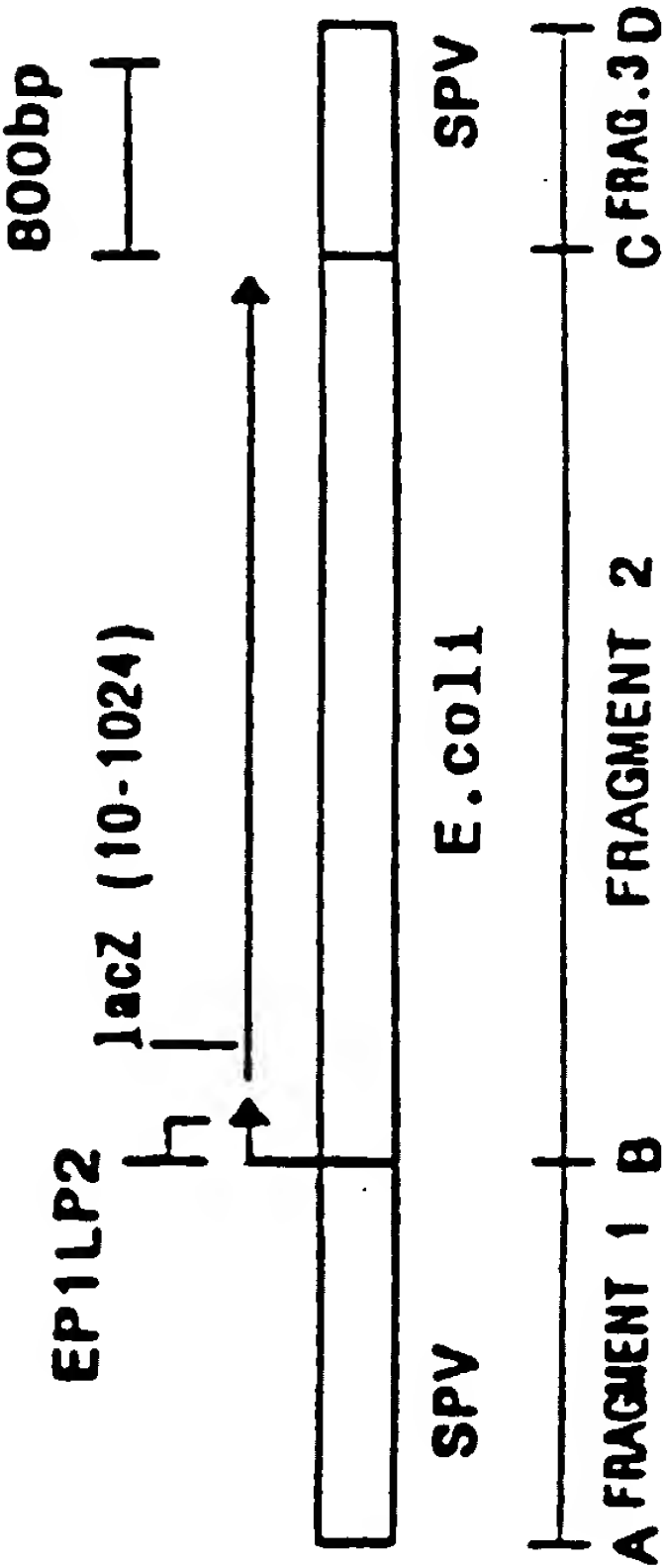
—

EcorV

	620	630	640	650	660	TERM
(C) W of O1L	CGVLFSYIKVNDK	IEHELEEMVDK	GTVP	SYLHLSINVIS	IILDDINGTR.	
(D) SPV EcoRV-EcoRI	SGIYFCQMRVHEQ	IELEIDELI	NGSMPVQLMHL	LLKVATI	ILEEIKEI.	→ EcoRI

FIGURE 3A

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DNA	ORIGIN	SITES	SIZE
VECTOR	pSP64	HindIII-BamHI	2972 BP
FRAGMENT 1	SPV HindIII	HindIII-AccI	2149 BP
FRAGMENT 2	pJF751	BamHI-PvuII	3002 BP
FRAGMENT 3	SPV HindIII	AccI-BglII	1484 BP

FIGURE 3B

JUNCTION A CAC ATA CGA TTT AGG=TGA CAC TAT AGA ATA=CAA GCT TTA TAC CAT=TAT AGA
 HindIII
 pSP64 ← SPV HindIII M

JUNCTION A (CONTINUED)

TAC ATT ACC=TTG TCC GAC GTG TAG=AAT TCA TGC CAA AGA=AGA ATT AAC TAA

[AccI] XbaI EP1
 GTA GTC=GAC TCT AGA AAA AAT=TGA AAA ACT ATT CTA=ATT TAT TGC ACG G

SPV HindIII M ←

JUNCTION B (CONTINUED)

BglII LP2 EcoRI BamHI
 AG=ATC TTT TTT TTT TTT=TTT TTT TTT GGC ATA=TAA ATG AAT TCG GAT=CCC GTC
 MET Asn Ser Asp Pro Val

lacZ(10)
 pJF751

FIGURE 3C

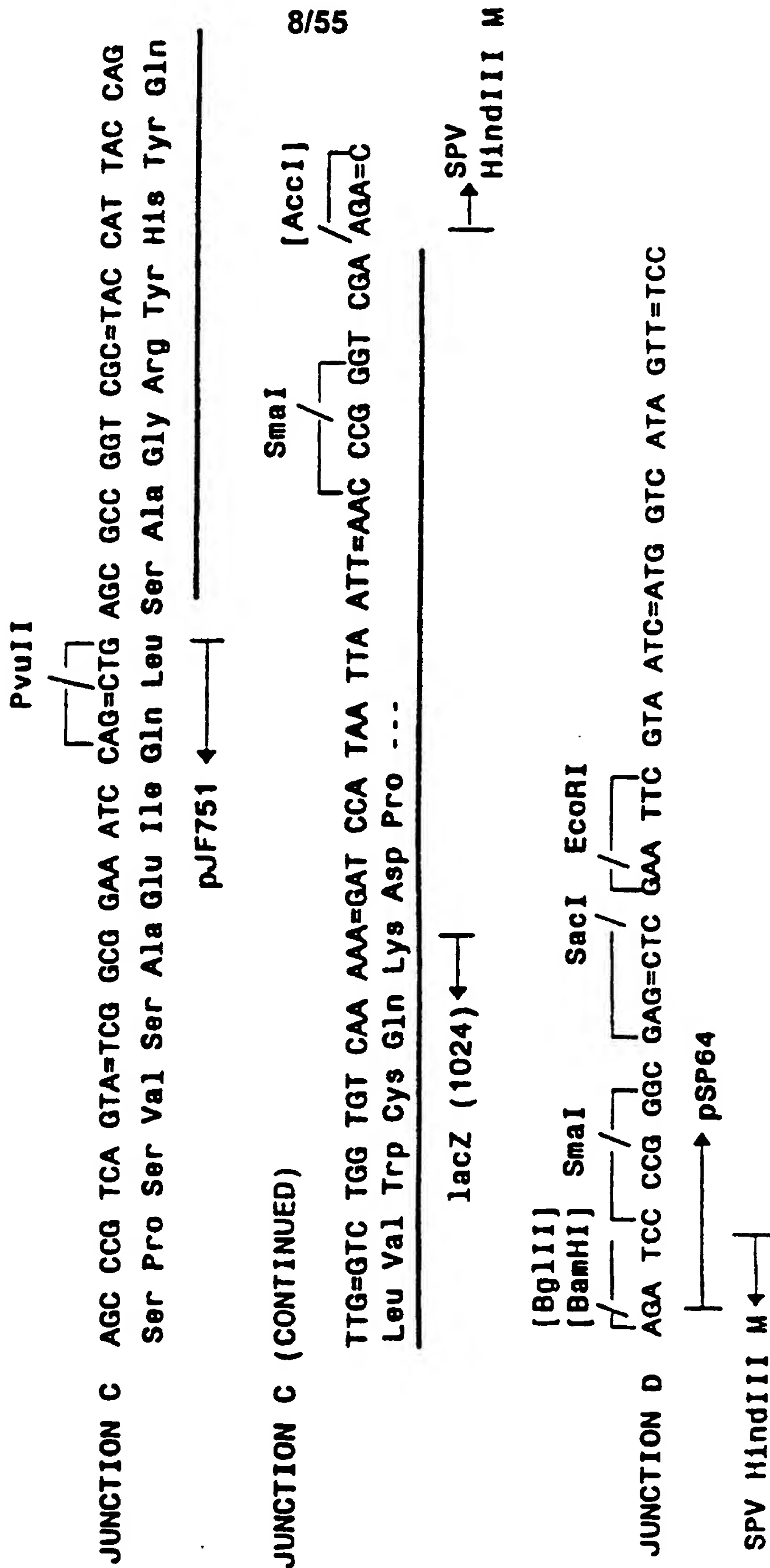
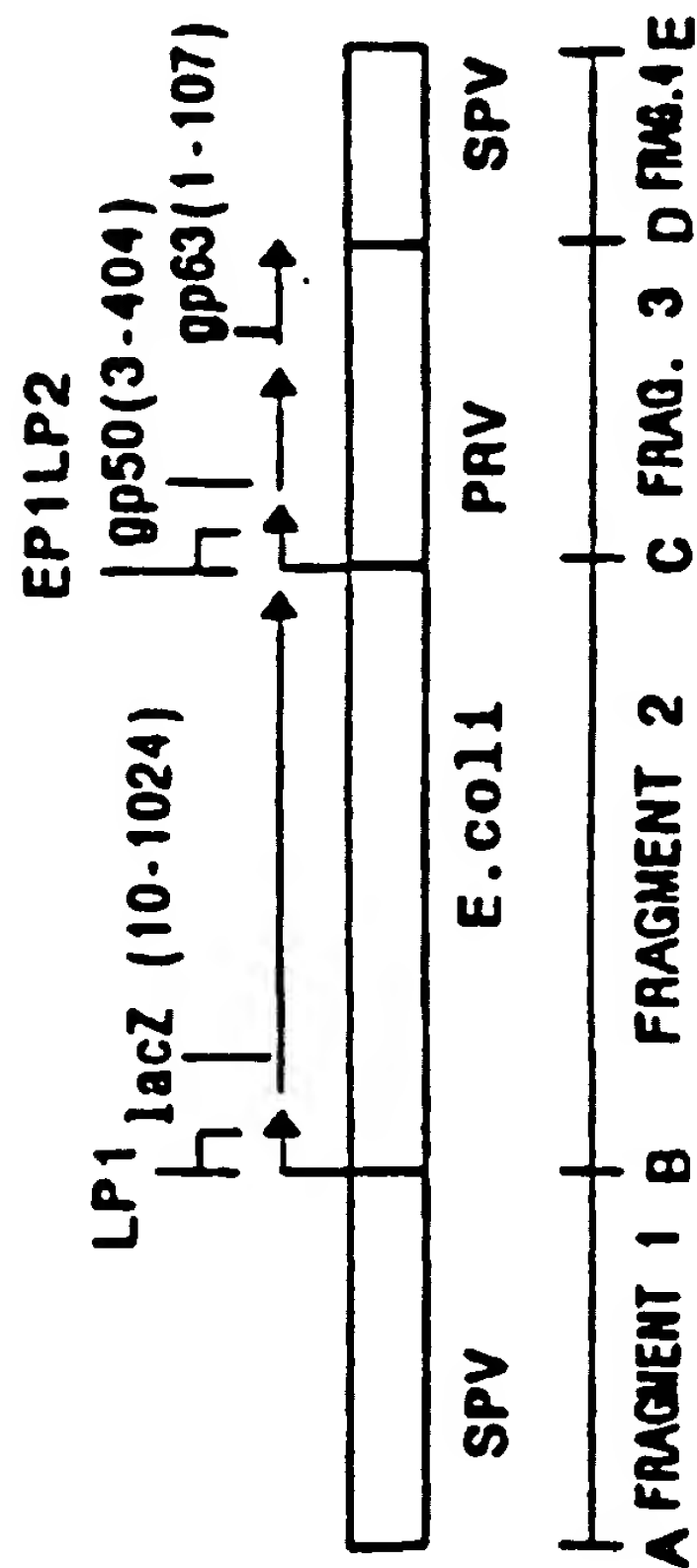


FIGURE 4A

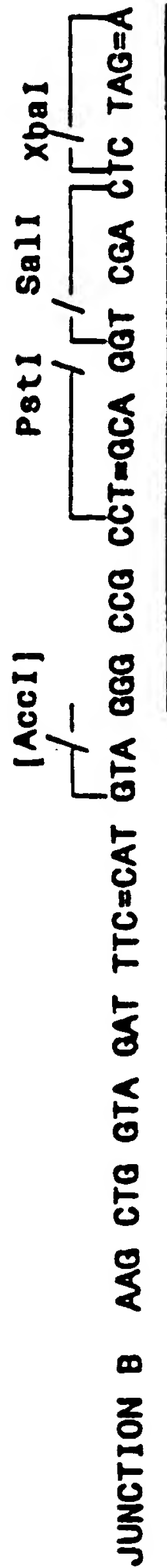
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DNA	ORIGIN	SLICES	SIZE
VECTOR	pSP64	HindIII-BamHI	~2972 BP
FRAGMENT 1	SPV HindIII M	HindIII-AccI	~2149 BP
FRAGMENT 2	pJF751	BamHI-PvuII	~3002 BP
FRAGMENT 3	PRV BamHI #7	ECORI*-StuI	~1558 BP
FRAGMENT 4	SPV HindIII M	AccI-BglII	~1484 BP

• INTRODUCED VIA CLONING

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SPV Hindi M

JUNCTION B (CONTINUED)

EcoRI / **BamHI**

LP1

AT TTC ATT TTG TTT=TTT TCT ATG CTA TAA=ATG AAT TCG GAT CCC=GTC GTT TTA CAA
MET Asn Ser Asp Pro Val Val Leu Gln

—►lacZ(10)

— — — — — ▶ PJF751

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FIGURE 4C

PvuII

JUNCTION C GTA TCG GCG GAA ATC CAG CTG AGC=GCC GGT CGC TAC CAT=TAC CAG TTG GTC TGG=TGT
Val Ser Ala Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys

pJF7514

PstI SalI

CAA=AAA GAT CCA TAA TTA=ATT AAC CCG GCC GCC=TGC AGG TGG ACT
Gln Lys Asp Pro ---

lacZ (1024)

XbaI

EP1

BglII

CT AGA AAA AAT TGA=AAA ACT ATT CTA ATT=TAT TGC ACG GAG ATC=

EcoRI

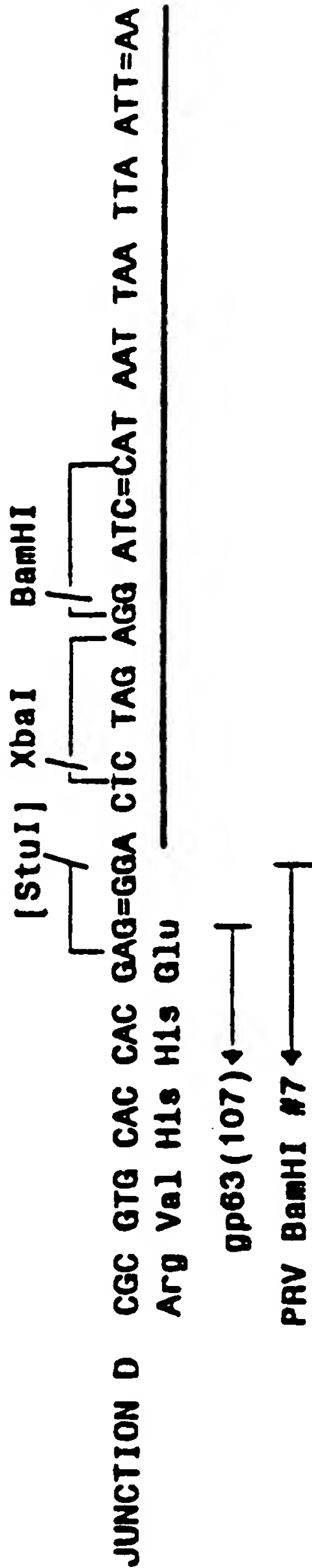
LP2

TT TTT TTT TTT TTT=TTT TTT GGC ATA TAA=ATG AAT TCG CTC GCA=GCG CTA TTG GCG GCG
MET Asn Ser Leu Ala Ala Leu Leu Ala Ala

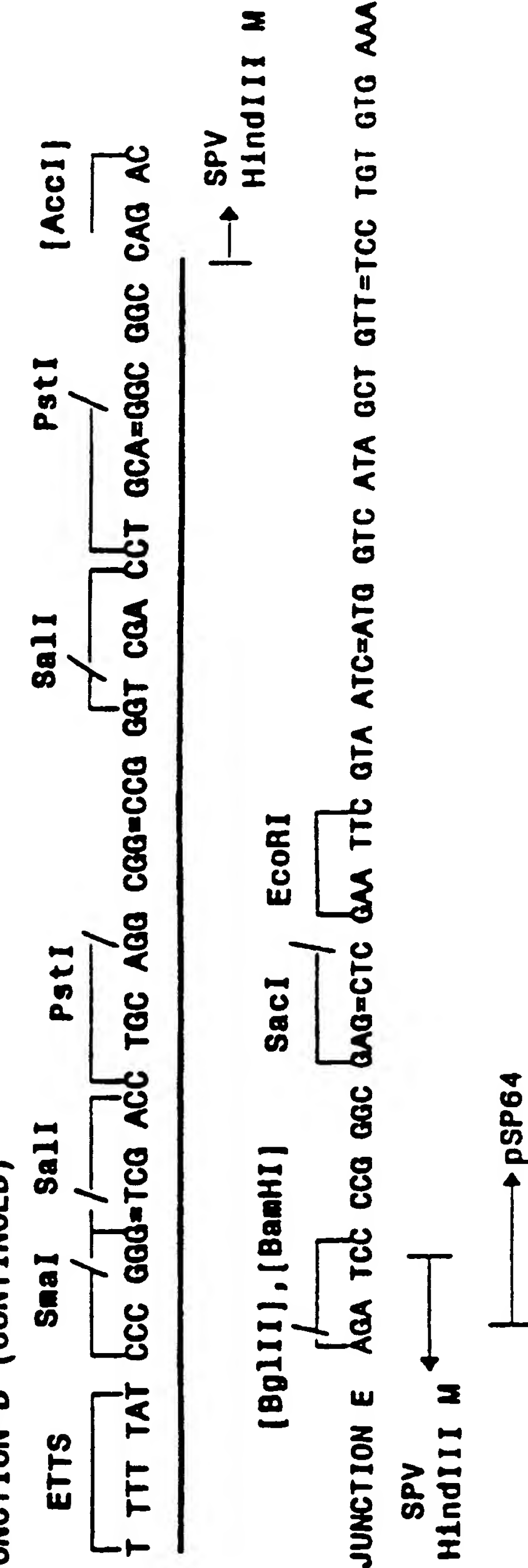
gp50(3)

PRV BamHI #7

FIGURE 4D



JUNCTION D (CONTINUED)



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FIGURE 5A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III—Bam HI	~2972 BP
Fragment 1	SPV HindIII M	Bgl II—Acc I	~1484 BP
Fragment 2	pJF751	Bam HI—Pvu II	~3002 BP
Fragment 3	PRV BamHI 2 & 9	Nco I—Nco I	~2378 BP
Fragment 4	SPV HindIII M	Acc I—Hind III	~2149 BP

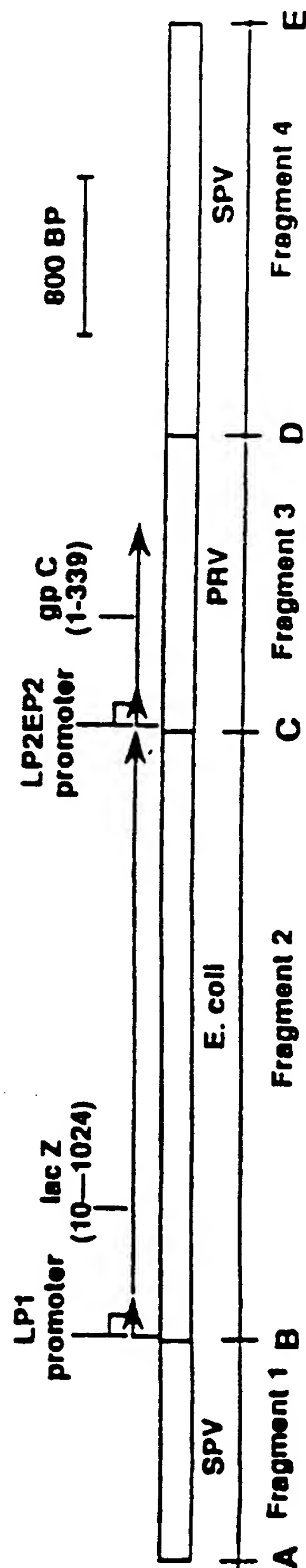
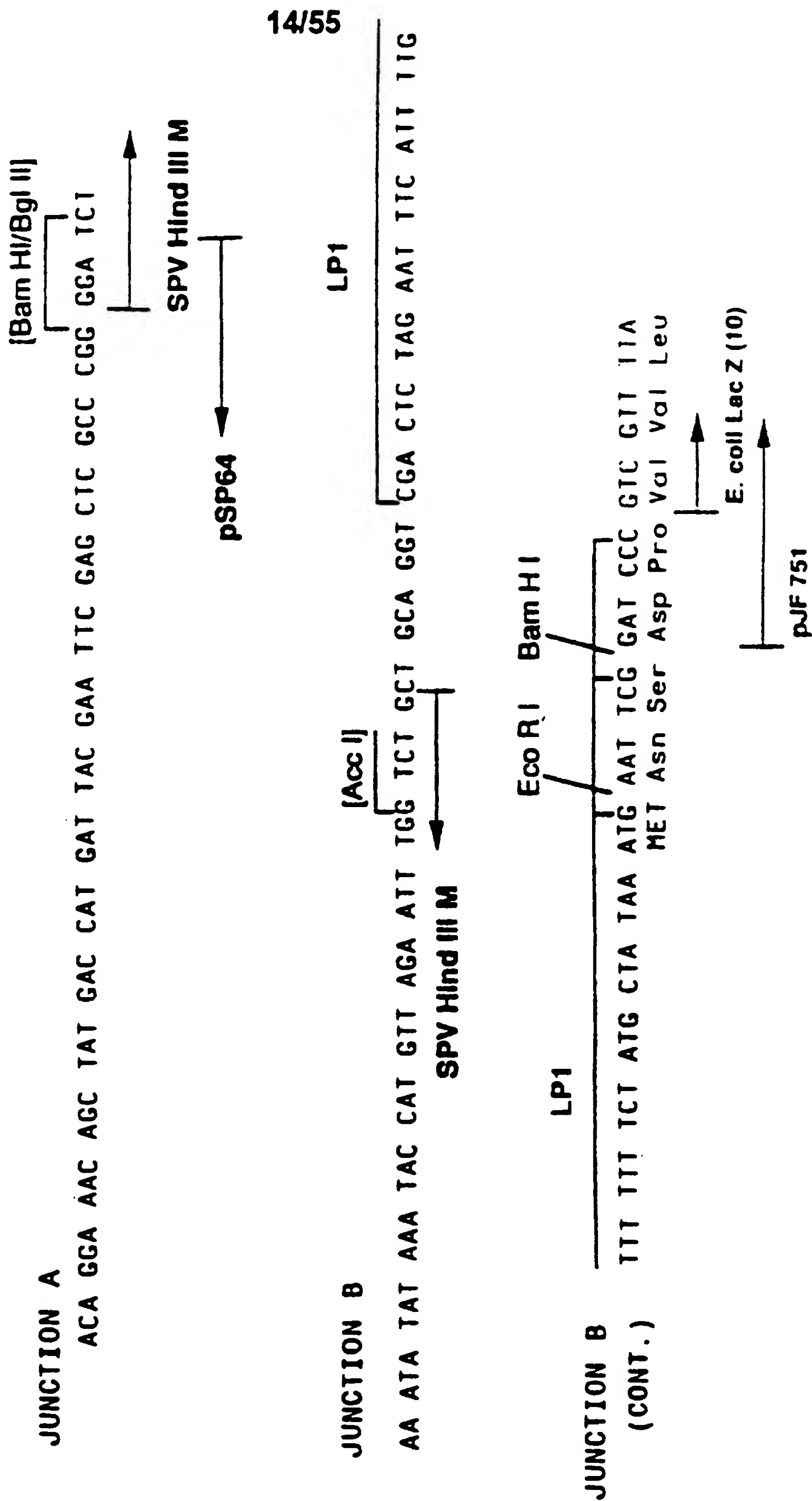


FIGURE 5B



JUNCTION C



JUNCTION C (CONT.)



2d7

CCA TAA TTA ATT AAC CCG GTC GAC TCT AGA TTT TTT TTT TTT TTT TTT TTT TTT TTT AAA
Pro .

JUNCTION C (CONT.)

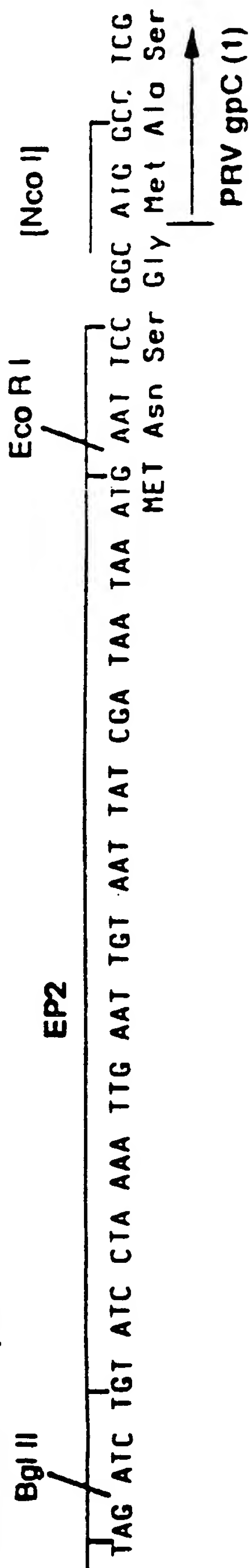
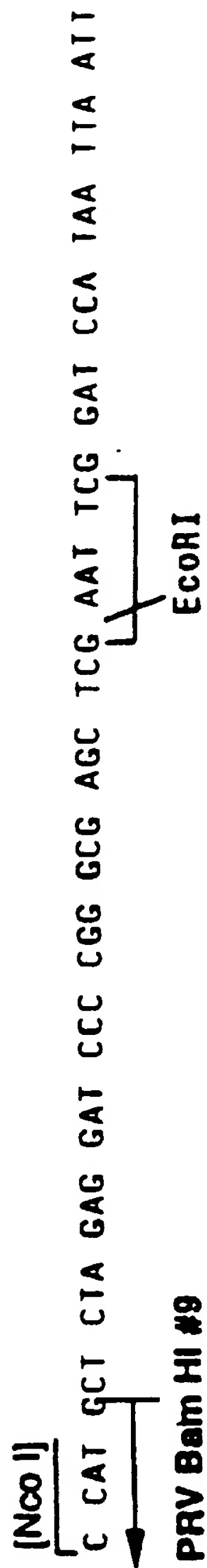


FIGURE 5D

JUNCTION D



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JUNCTION D (CONT.)



JUNCTION E

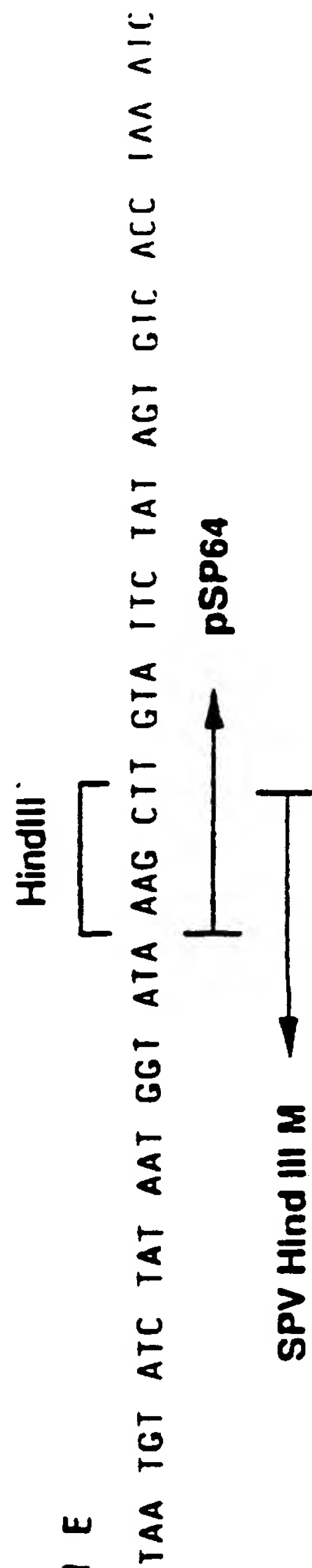
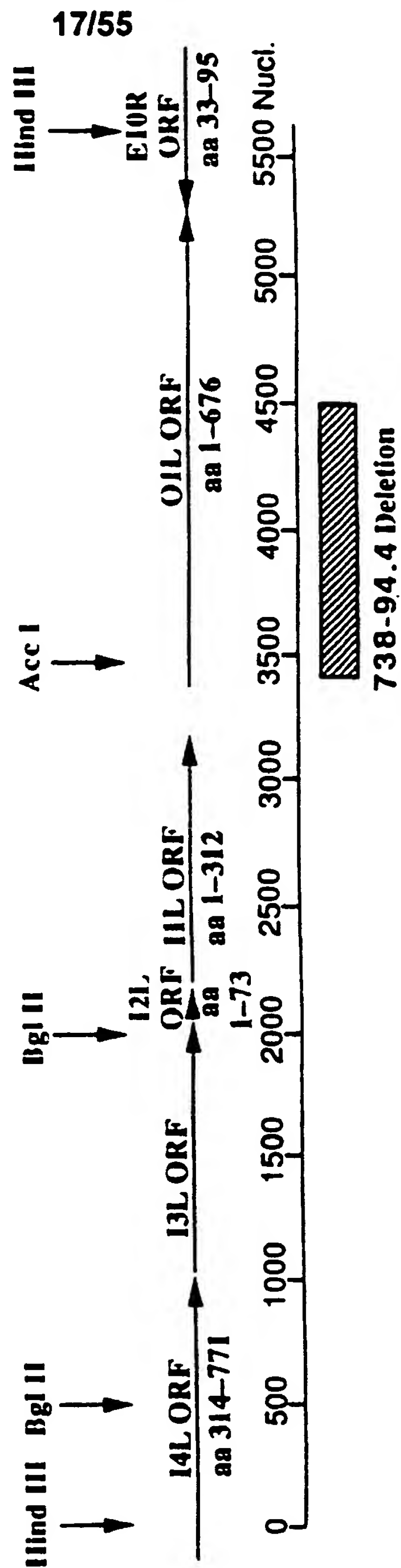


FIGURE 6



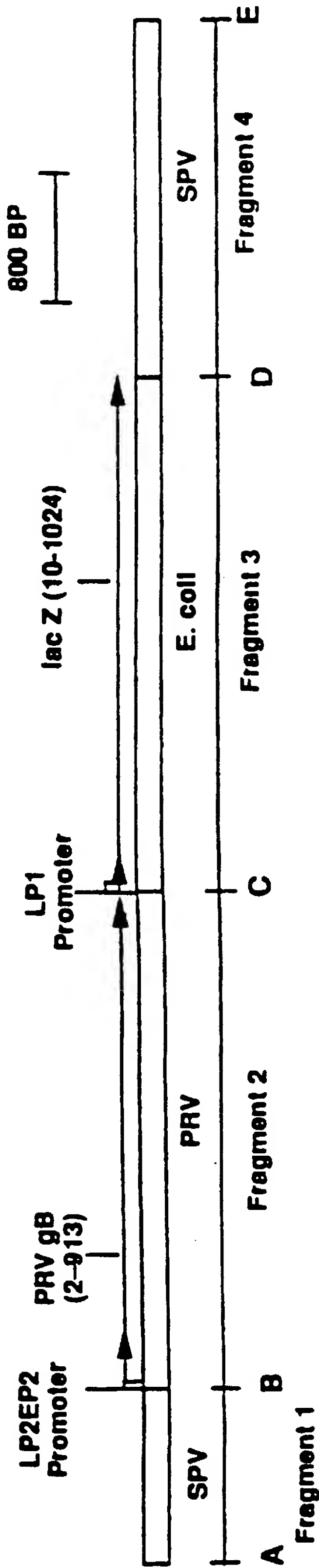
SEQ ID NO. 195

SEQ ID NO. 189

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FIGURE 7A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III-Bam HI	~2972 BP
Fragment 1	SPV Hind III M	Bgl II-Acc I	~1484 BP
Fragment 2	PRV Kpn I C	Sma I-Sac I	~3500 BP
Fragment 3	pJF751	Bam HI-Pvu II	~3010 BP
Fragment 4	SPV Hind III M	Acc I-Hind III	~2149 BP



Junction A

ACA GGA AAC AGC TAT GAC CAT GAT TAC GAA TTC GAG CTC GCC CGG GGA TCT

SPV Hind III M

pSP64

Junction B

LP2

[Acc I] Not I Sal I Xba I

GTA TTGC GGC CGC CTG CAG GTC GAC TCT AGA TTT TTT TTT TTT TTT TTT TTT TTT TTT AAA

SPV Hind III M

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Junction B
Continued

	Bgl II												EP2											
	TAG	ATC	TGT	ATC	CIA	AAA	TIG	AAT	TGT	AAT	TAT	CGA	TAA	TAA										

Junction B Continued

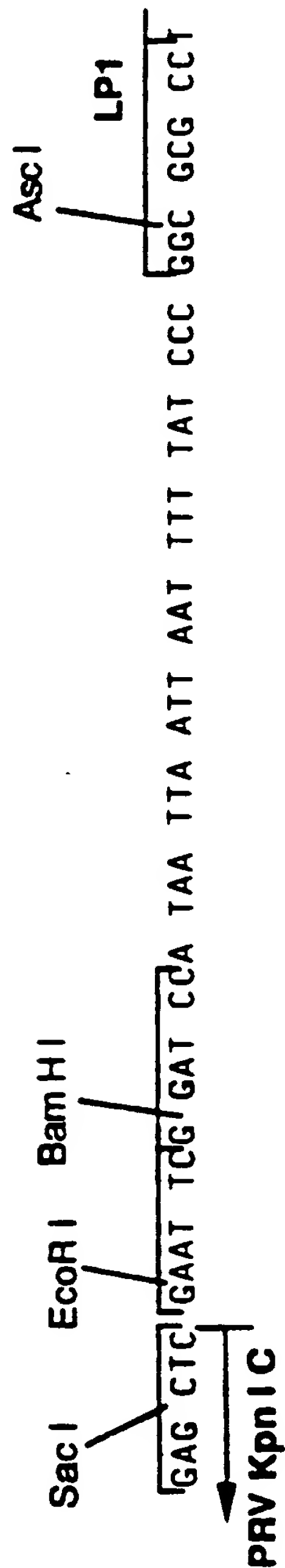
EcoRI

ATGAA TTC ACC CGC TGG TGG CGG TCT TIG GCG CGG GCC CCG TGG GCA TCG GGC GGC CCG GAA CCA CCG
 Synthetic PRV gB (2-16)
 N-terminal (Synthetic)

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FIGURE 7C

Junction C



Junction C Continued

LP1

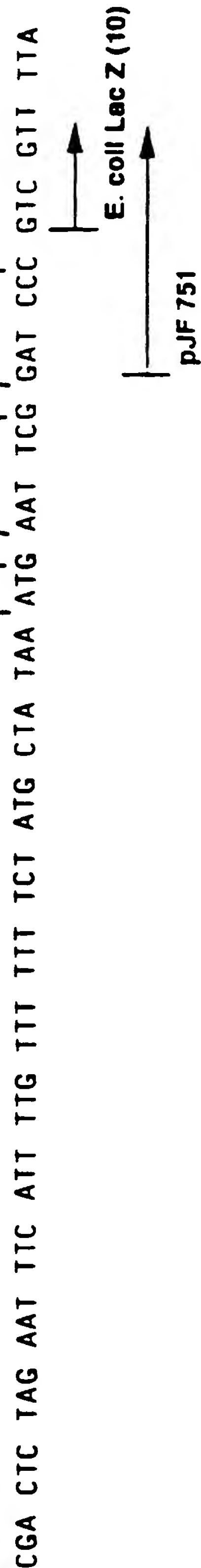


FIGURE 7D

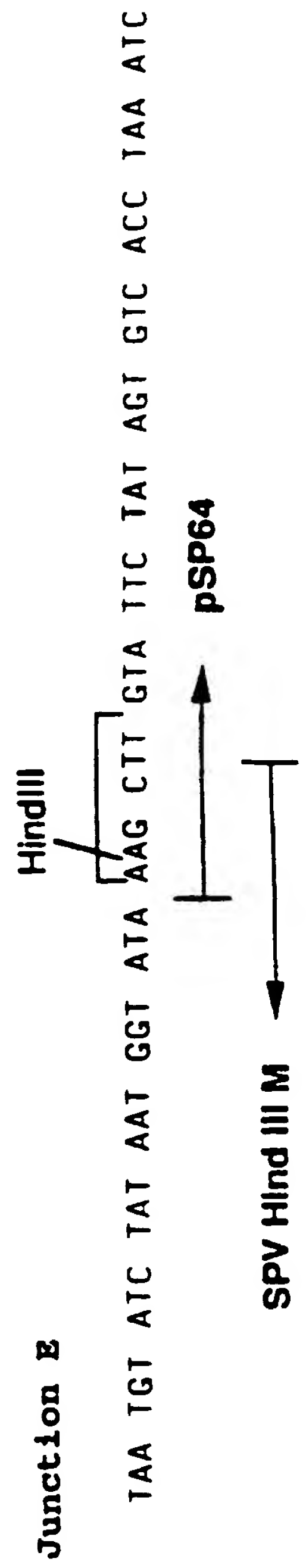
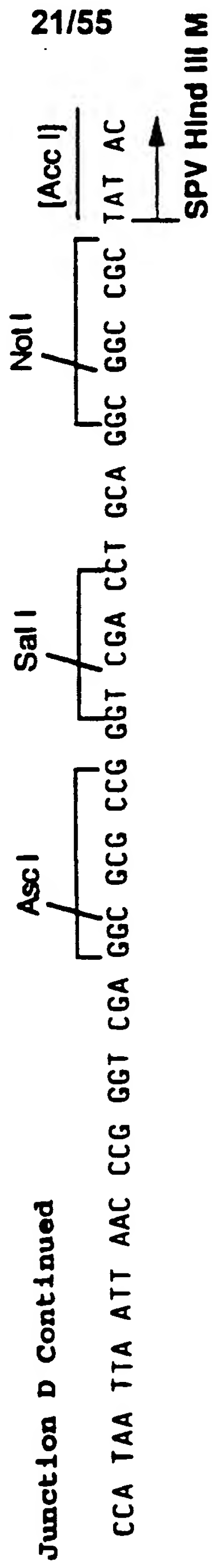
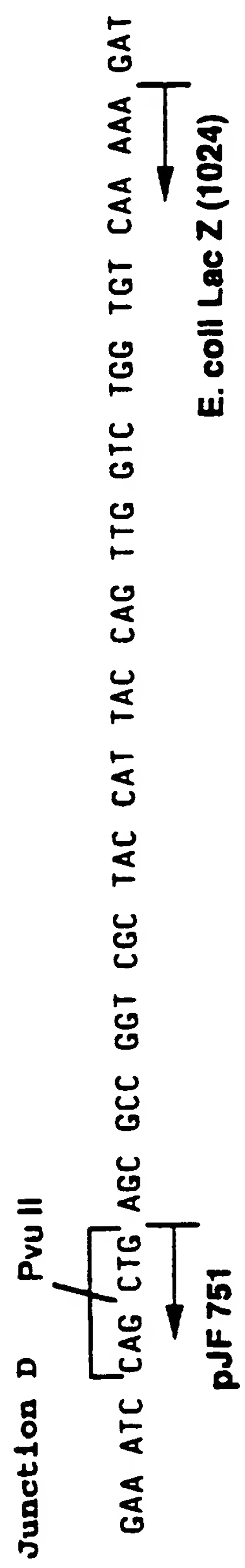
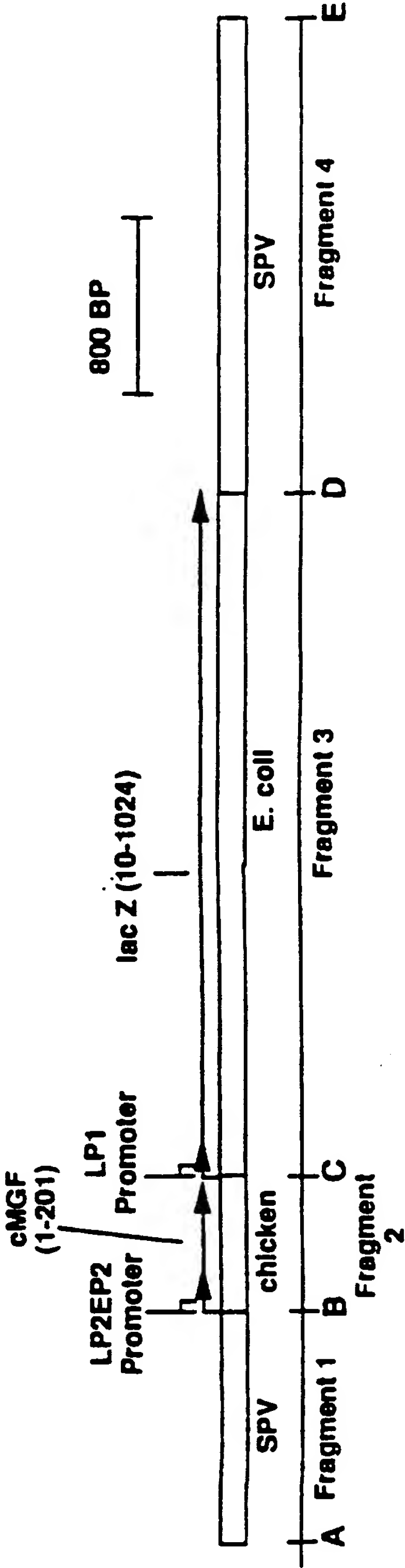


FIGURE 8A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III-Bam HI	~2972 BP
Fragment 1	SPV Hind III M	Bgl II-Acc I	~1484 BP
Fragment 2	chicken MGF	EcoR I†-BamH I†	~640 BP
Fragment 3	pJF751	Bam HI-Pvu II	~3002 BP
Fragment 4	SPV Hind III M	Acc I-Hind III	~2149 BP

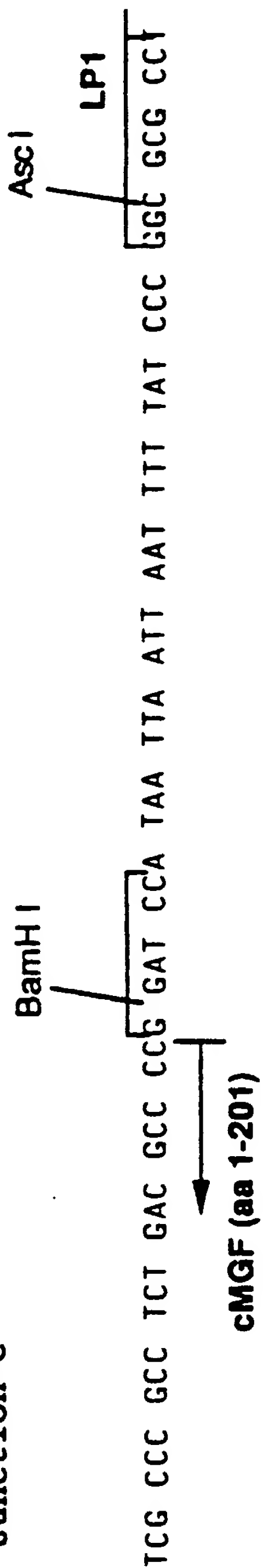
†Restriction sites introduced by PCR cloning



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FIGURE 8C

Junction C



Junction C Continued

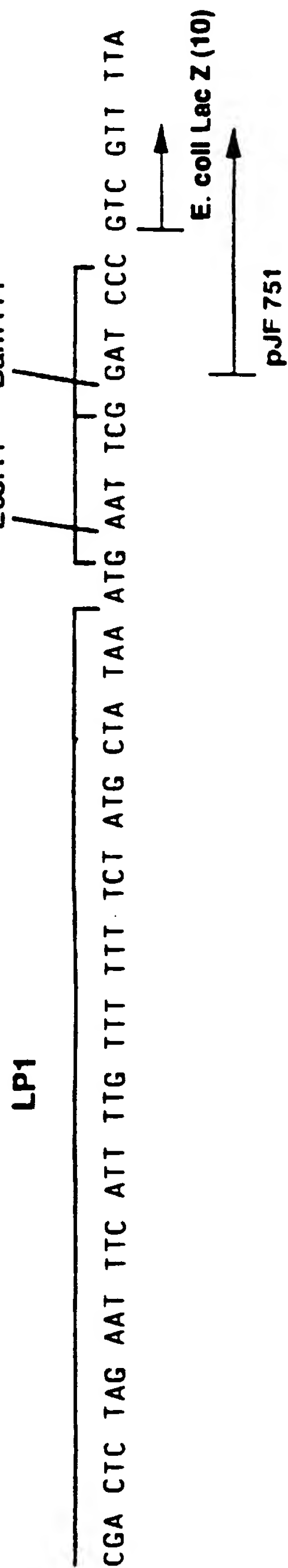


FIGURE 8D

Junction D Pvu II

GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT CAA AAA GAT
 pJF 751 E. coli Lac Z (1024)

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Junction D Continued

CCA TAA TTA ATT AAC CCG GGT CGA GGC GCG CCG GGT CGT CGA CCT GCA GGC GGC CGC TAT AC
 Asc I Sal I Not I [Acc I] SPV Hind III M

Junction E

TAA TGT ATC TAT AAT GGT ATA AAG CTT GTA TTC TAT AGT GTC ACC TAA ATC
 Hind III pSP64 SPV Hind III M

FIGURE 9A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III-Bam HI	~2972 BP
Fragment 1	SPV Hind III M	Bgl II-Acc I	~1484 BP
Fragment 2	chicken IFN	EcoR I†-Bgl II†	~577 BP
Fragment 3	pJF751	Bam HI-Pvu II	~3002 BP
Fragment 4	SPV Hind III M	Acc I-Hind III	~2149 BP

†Restriction sites introduced by PCR cloning

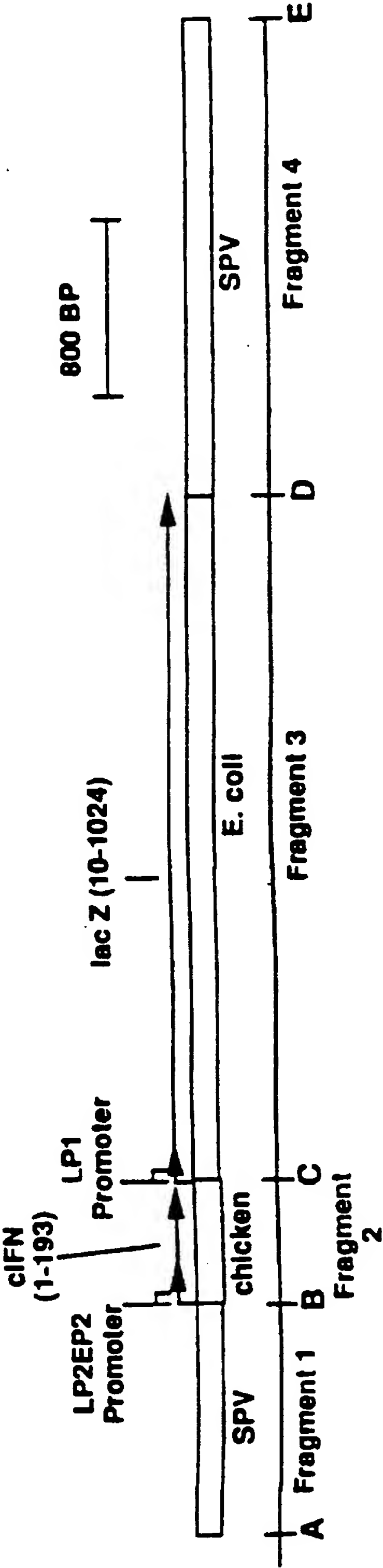


FIGURE 9B

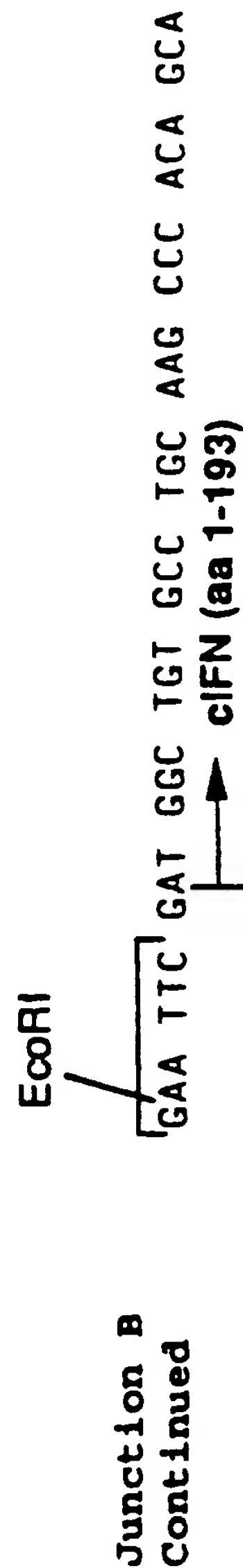


FIGURE 9C

Junction C

[Bgl II, Bam HI]

CTT AGC CCC AAA CGC ACC TCA GAT CCA TTA ATT AAT TTT TAT CCC GGC GCG CCT

cIFN (aa 1-193)

Asc I

LP1

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Junction C Continued

LP1

CGA CTC TAG AAT TTC ATT TIG TTT TTT TCT ATG CTA TAA ATG AAT TCG GAT CCC GTC GTT TTA

EcoRI BamHI

E. coli Lac Z (10)

pJF 751

FIGURE 9D

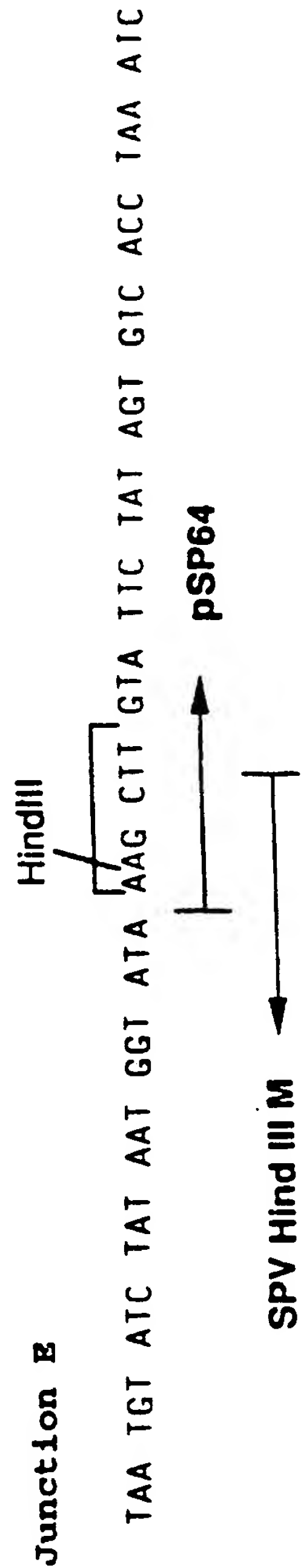
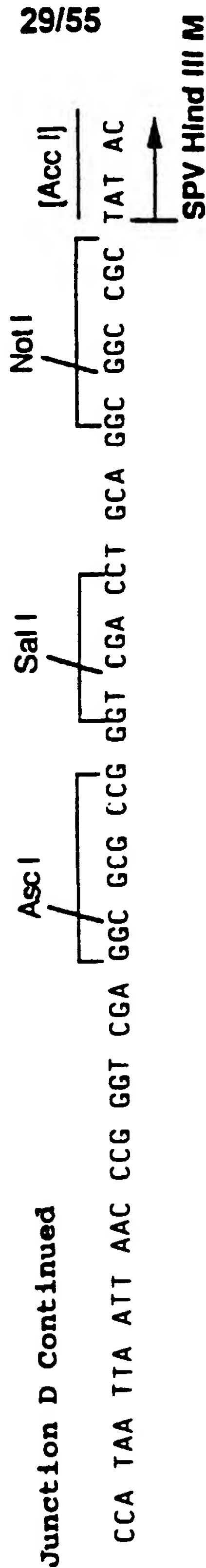
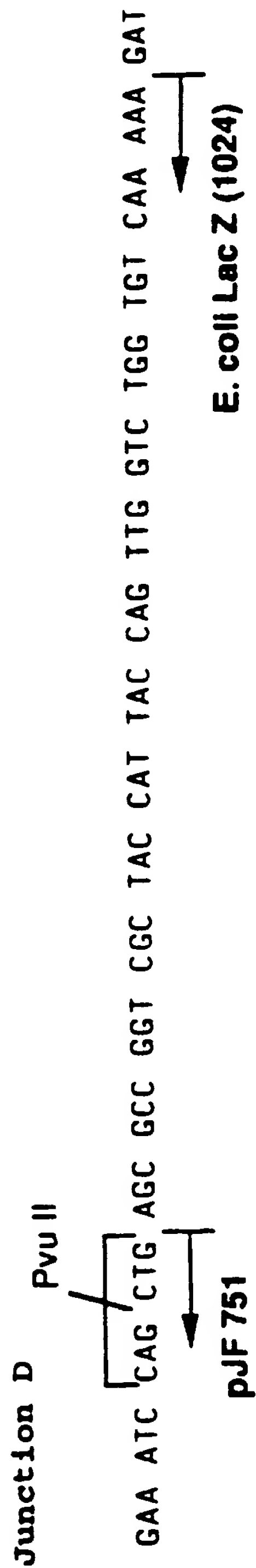


FIGURE 10A

DNA	Origin	Sites	Size
Vector	pSP65	Hind III-Sph I	~2519 BP
Fragment 1	SPV Hind III M	Sph I-Bgl II†	~855 BP
Fragment 2	pJF751	Bam HI-Pvu II	~3002 BP
Fragment 3	SPV Hind III M	Sal I†-Hind III	~1113 BP

†Restriction sites introduced by PCR cloning

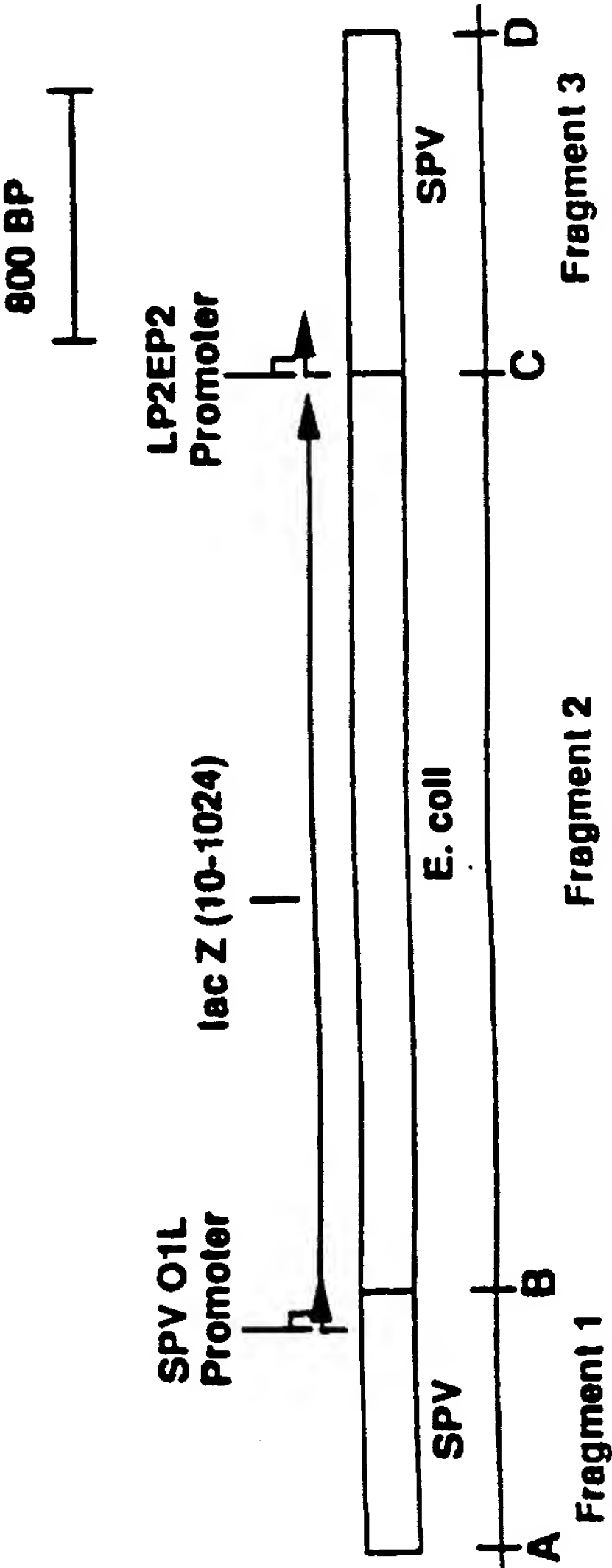
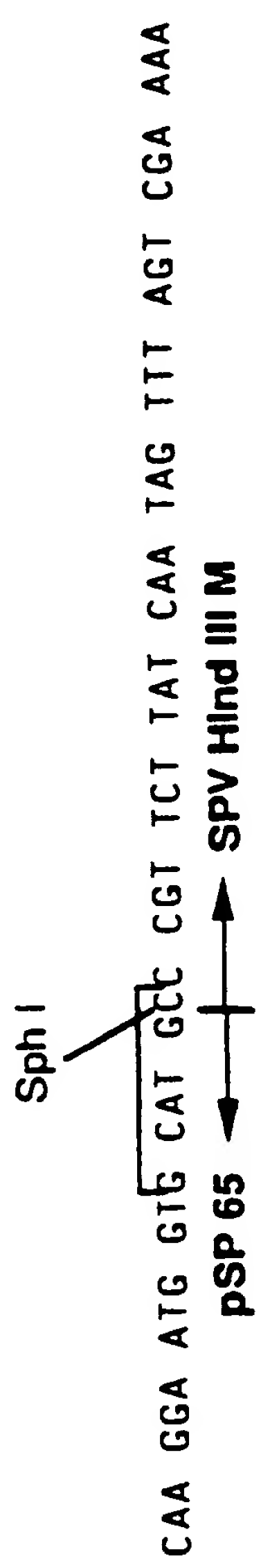
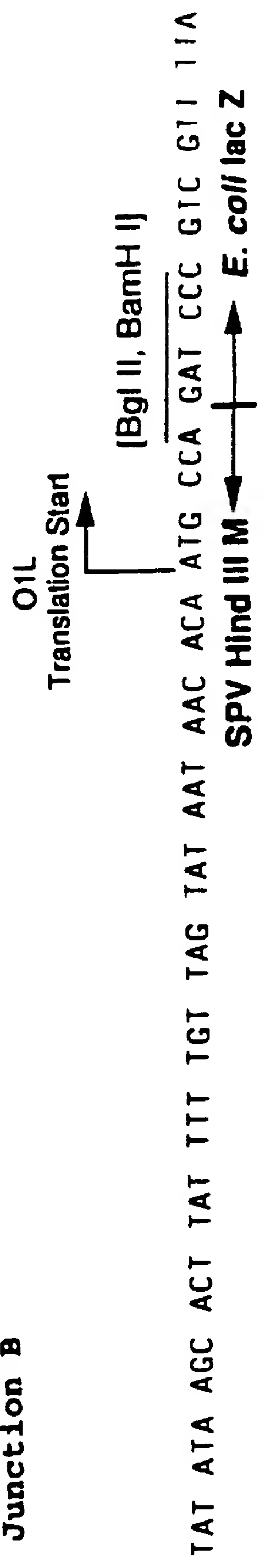


FIGURE 10B

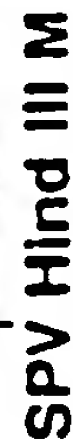
Junction A



Junction B



Junction C



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FIGURE 10D

Junction D

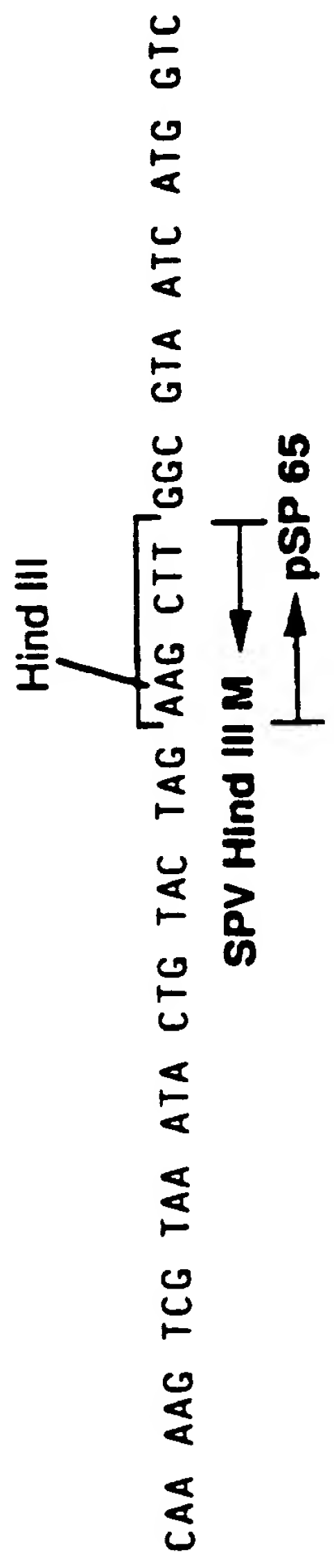
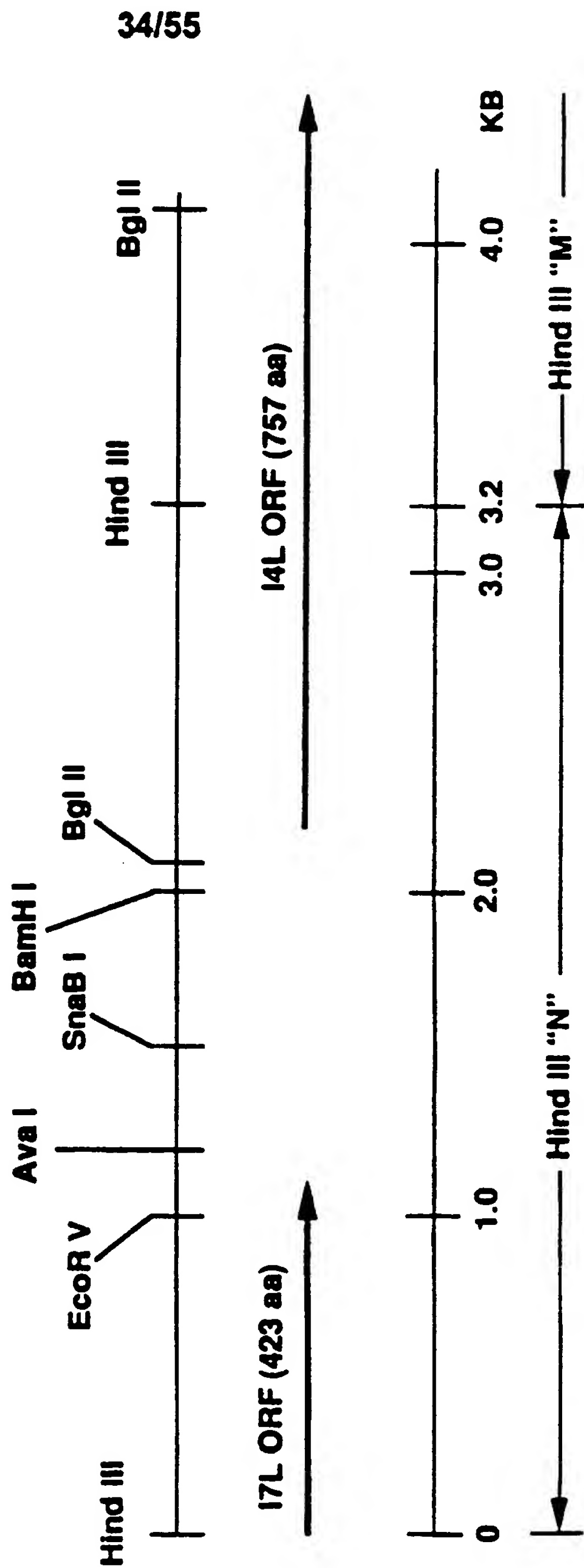


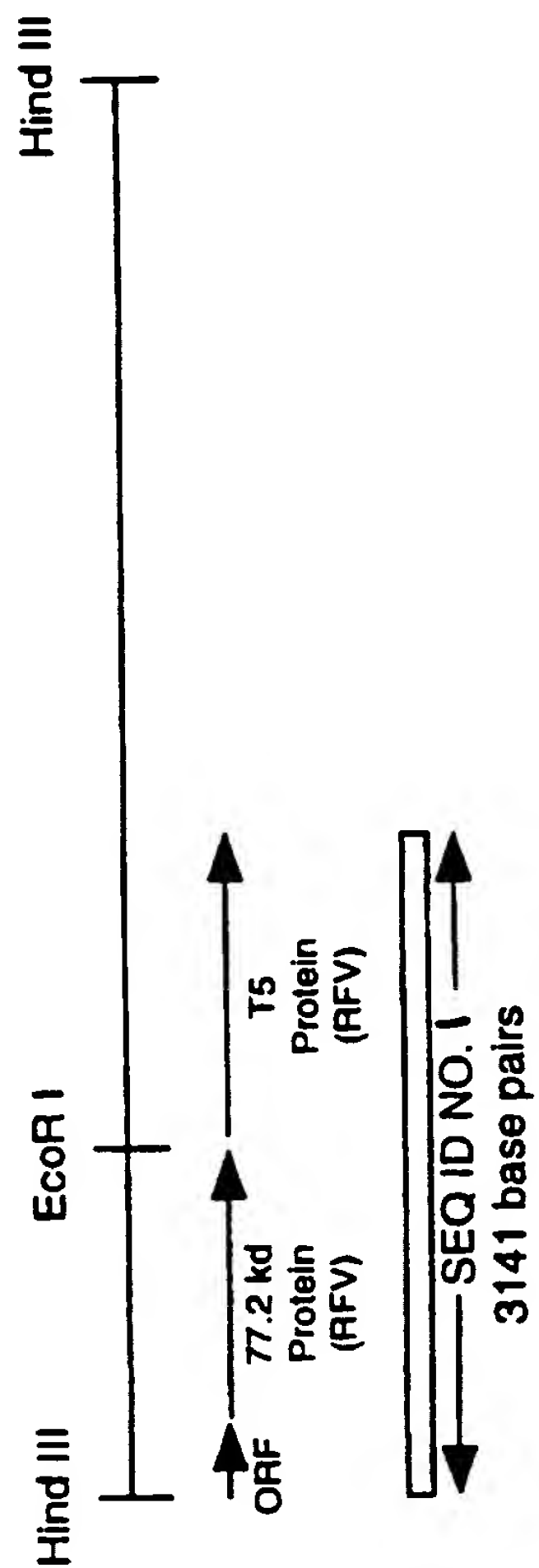
FIGURE 11A



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FIGURE 11B

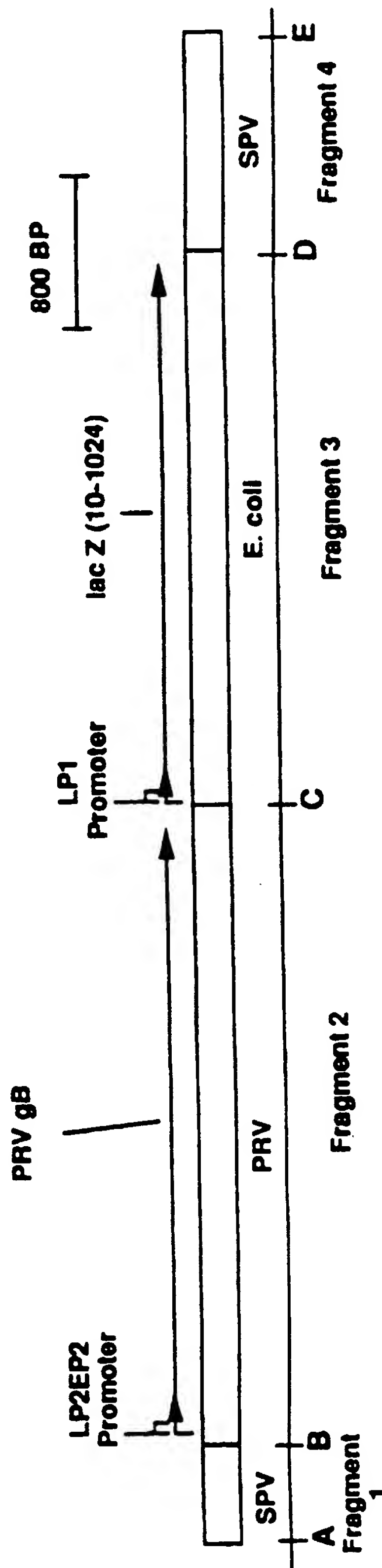
SPV HindIII K Genomic Fragment
6.7 kb



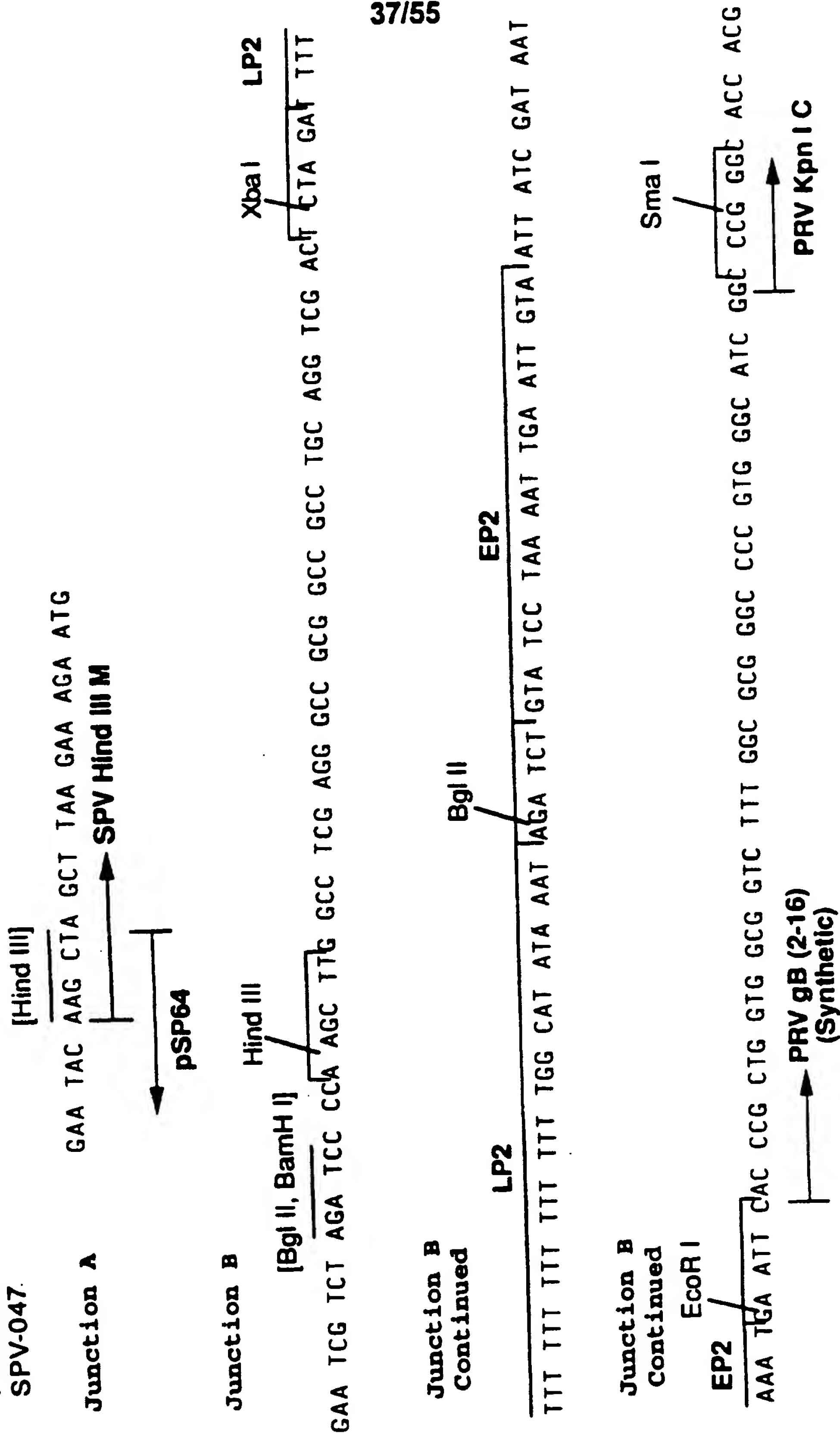
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FIGURE 12A

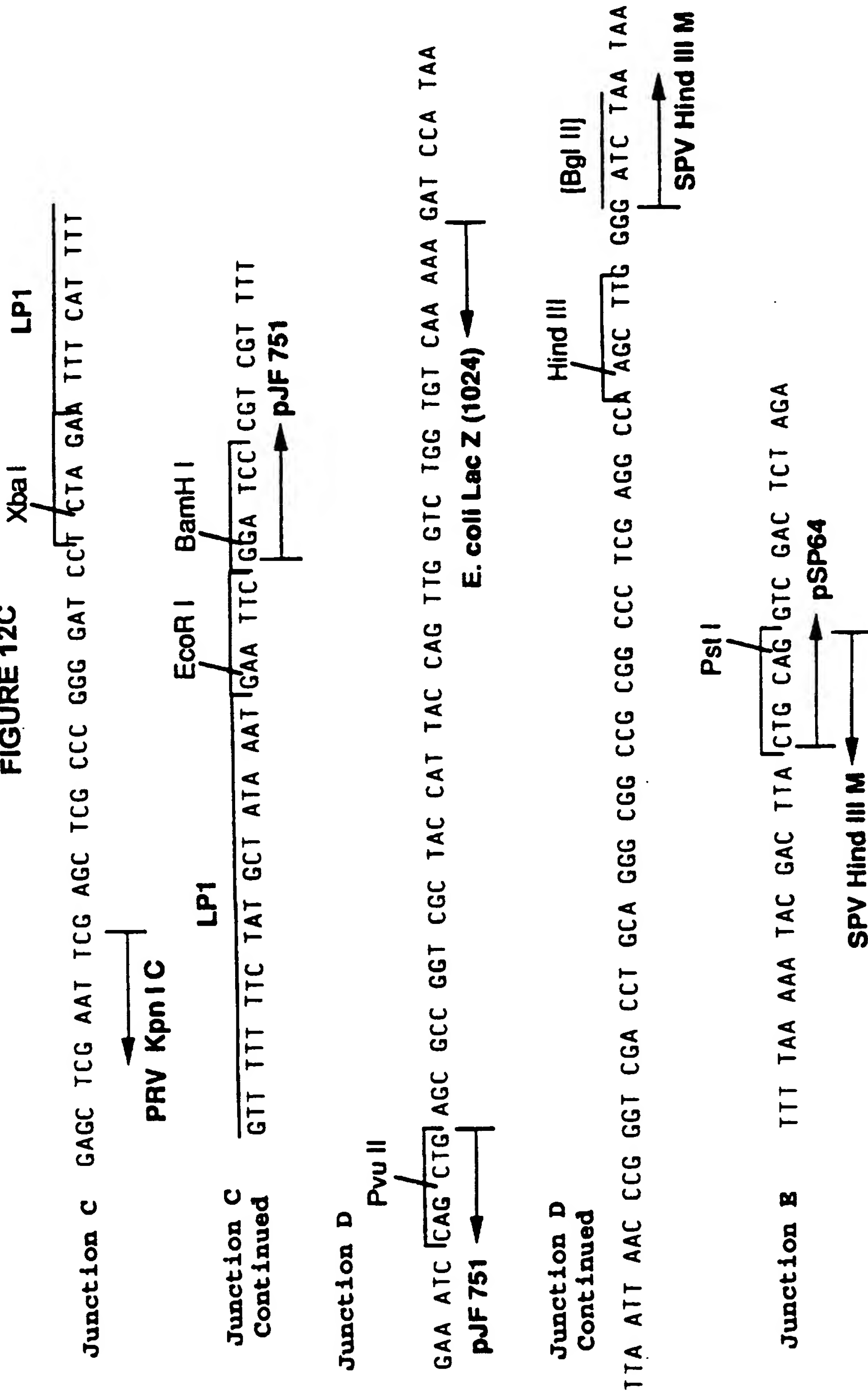
DNA	Origin	Sites	Size
Vector	pSP64	Pst I-Hind III	~2986 BP
Fragment 1	SPV Hind III M	Hind III-Bgl II	~542 BP
Fragment 2	PRV Kpn I C	Sma I-Sac I	~3500 BP
Fragment 3	pJF751	Bam HI-Pvu II	~3010 BP
Fragment 4	SPV Hind III M	Bgl II-Pst I	~1180 BP



**779-94.31
SPV-047.**



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FIGURE 13A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III-BamH I	~2972 BP
Fragment 1	SPV Hind III M	Bgl II-Acc I	~1484 BP
Fragment 2	PRV BamH I #7	EcoR I-Stu I	~1552 BP
Fragment 3	SPV Hind III M	Acc I-Nde I	~48 BP
Fragment 4	PRV Kpn I C	Sma I-EcoR I	~3500 BP
Fragment 5	pJF751	Bam HI-Pvu II	~3010 BP
Fragment 6	SPV Hind III M	Nde I-Hind III	~1560 BP

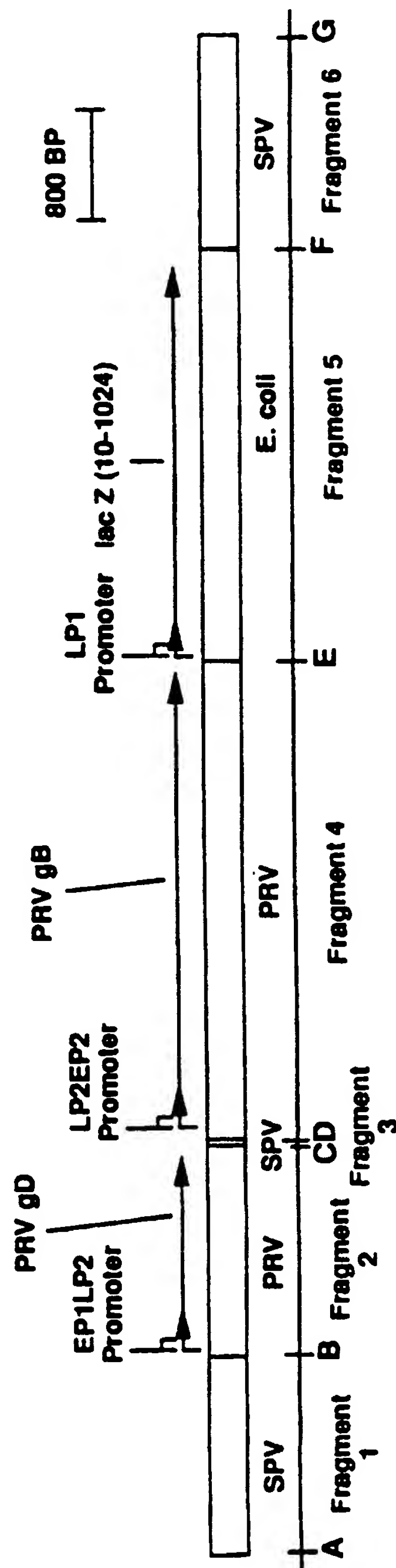


FIGURE 13B

789-41.7.
SPV-052.

[Bgl II, BamH I]

Junction A
GCC CGG GGA TCT TGA AGA TGA ATG CAT
SPV Hind III M

pSP64

Junction B
Pst I

[Acc I]

EP1LP2

ATT TGG TCT GCT GCA GGT CGA CTC TAG AAA AAA TTG AAA AAC TAT TCT AAT TTA TTG CAC
SPV Hind III M

EcoRI

Junction B
continued

EP1LP2

GGA GAT CTT TTT TTT TTT TTT TTT TGG CAT ATA AAT GAA TTC GCT CGC AGC GCT
PRV Bam HI #7

[Stu I]

Junction C
GCG TGC ACC ACG AGG GAC TCT AGA GGA TCC ATA ATT AAT TAA TTA ATT TTT
PRV Bam HI #7

Pst I

[Acc I]

Junction C
continued
ATC CCG GGT CGA CCT GCA GCC TAC ATG GAA ATC TAC CAG
SPV Hind III M

FIGURE 13C

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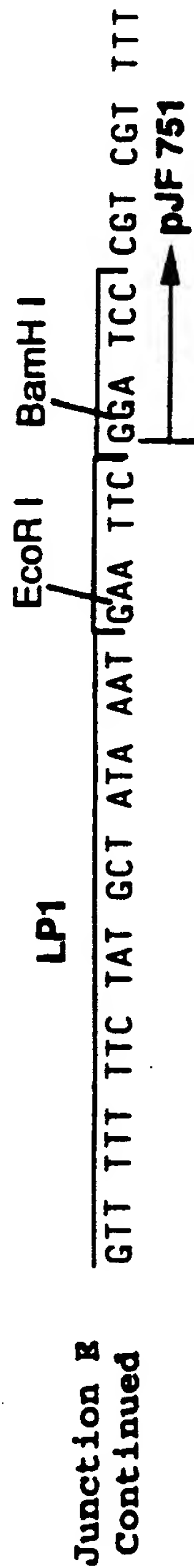
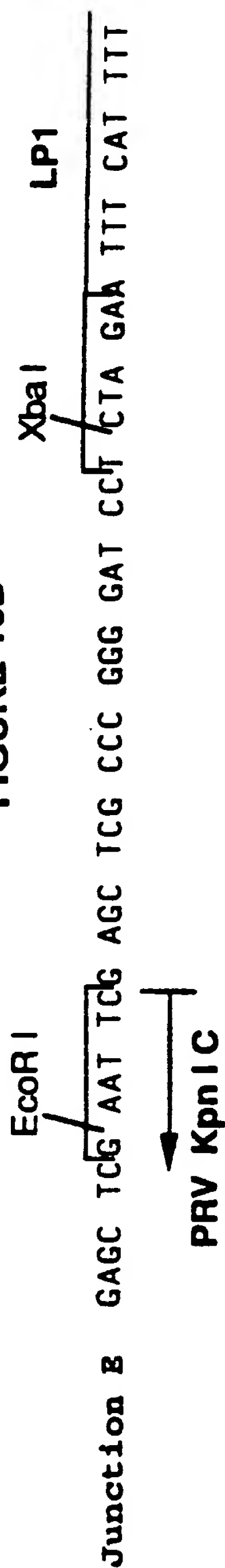
Junction D continued

EP2
 TAA TAA ATG AAT TCA CCC GCT GGT GGC GGT CTT TGG CGC GGG CCC CGT GGG CAT CGG
 EcoRI
 SmaI
 PRV gB (2-16)
 (Synthetic)
 PRV KpnI C

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FIGURE 13D



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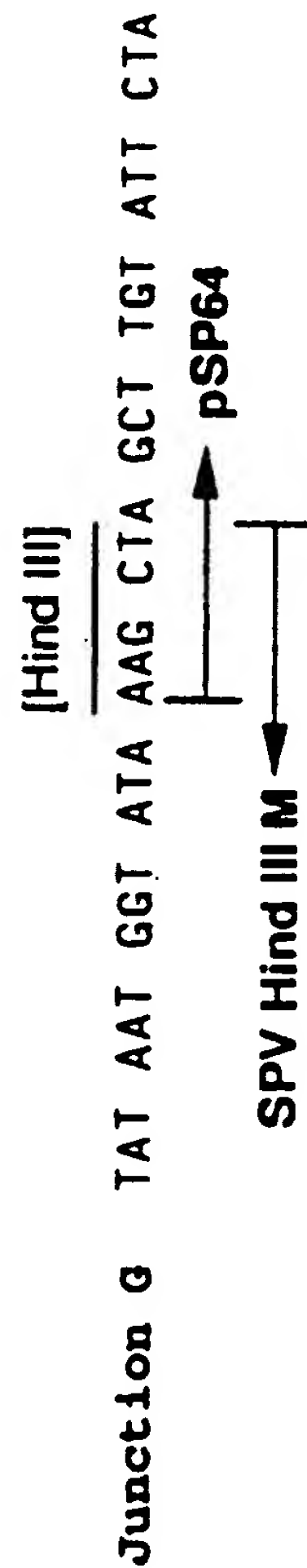
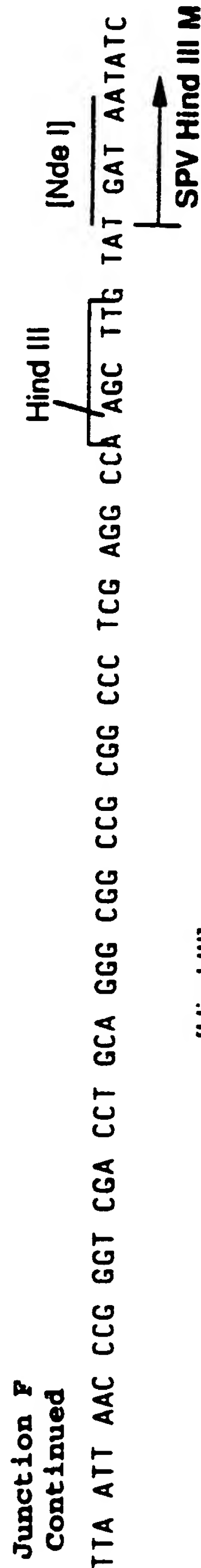
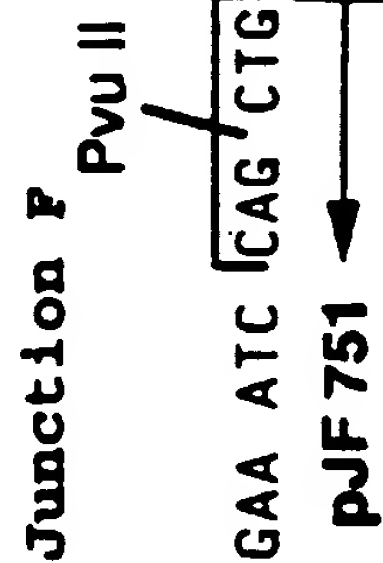
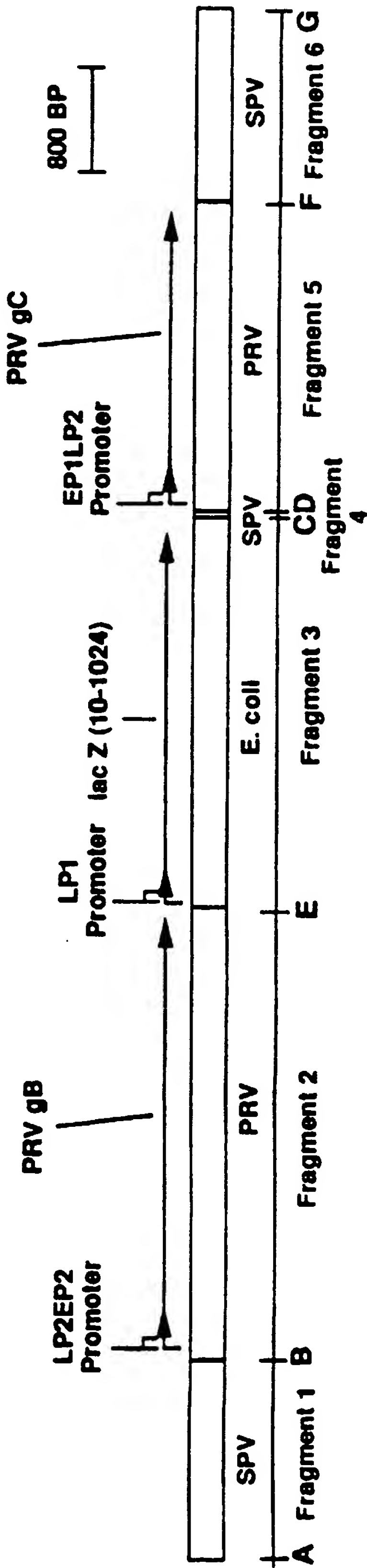


FIGURE 14A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III-BamH I	~2972 BP
Fragment 1	SPV Hind III M	Hind III-Nde I	~1560 BP
Fragment 2	PRV Kpn I C	Sma I-EcoR I	~3500 BP
Fragment 3	pJF751	Bam HI-Pvu II	~3010 BP
Fragment 4	SPV Hind III M	Nde I-Acc I	~48 BP
Fragment 5	PRV BamH I #2 & #9	Nco I-Nco I	~2378 BP
Fragment 6	SPV Hind III M	Acc I-Bgl II	~1484 BP

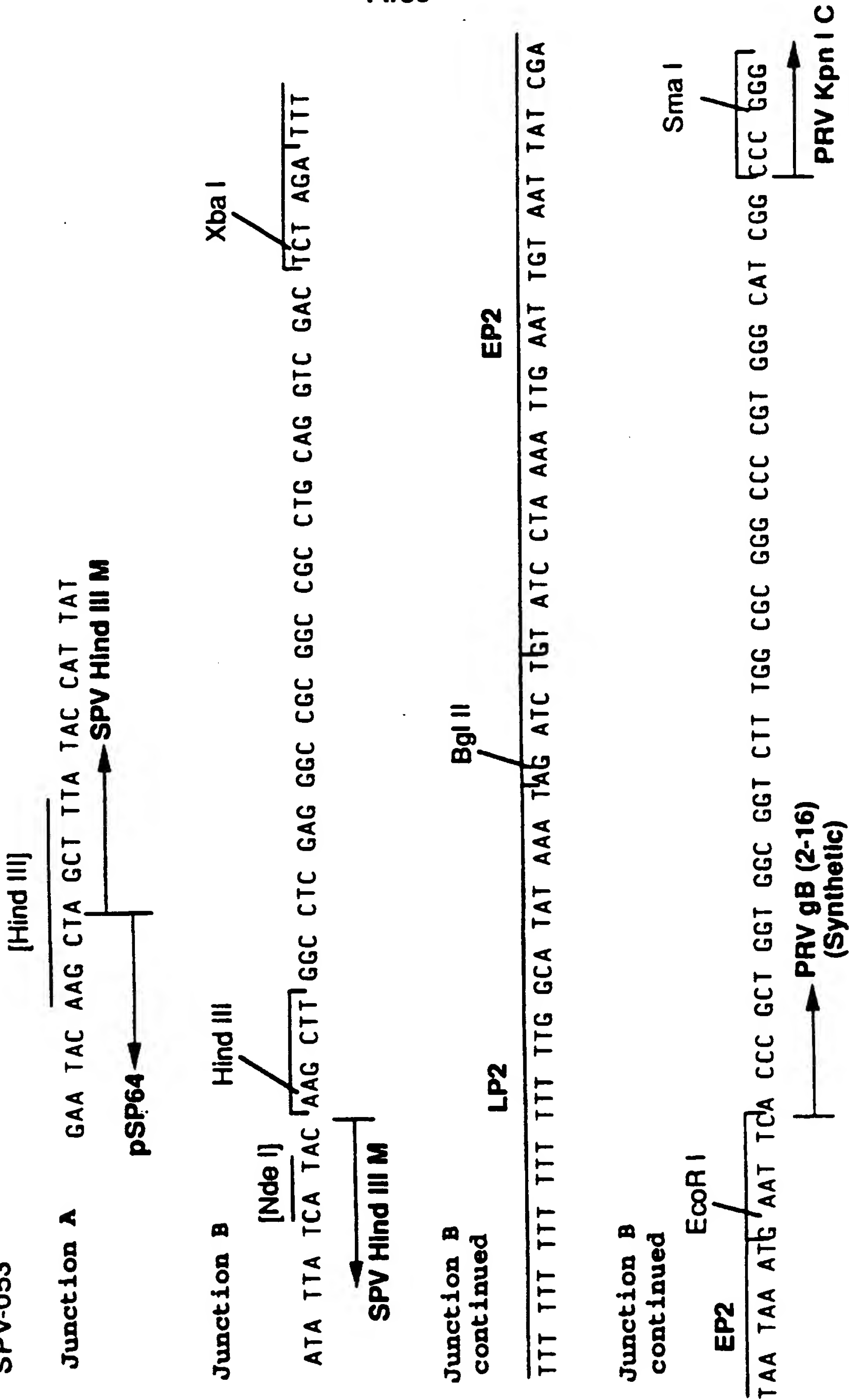


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PCT/US97/12212

FIGURE 14B

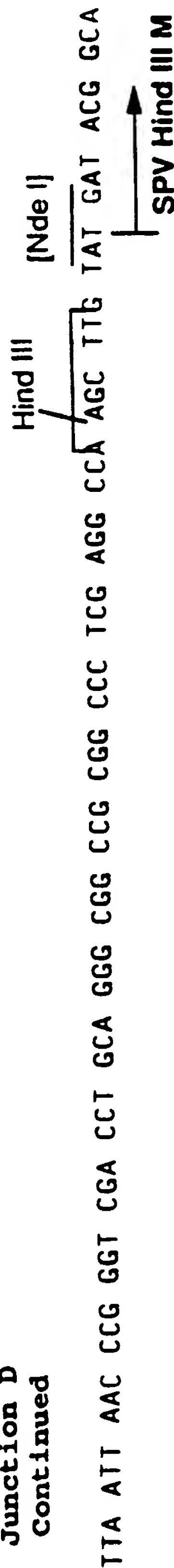
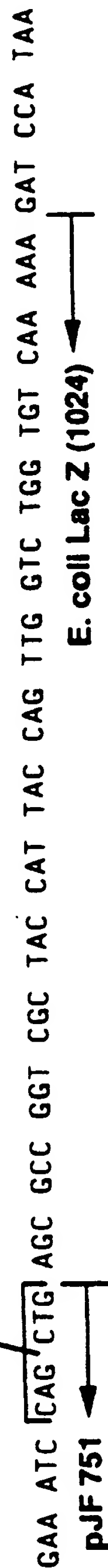
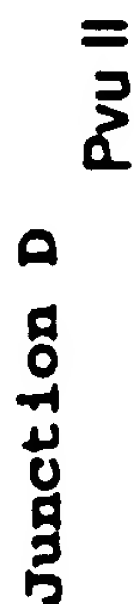
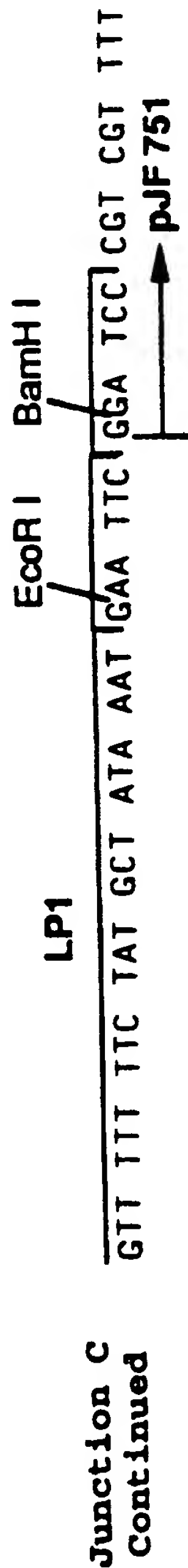
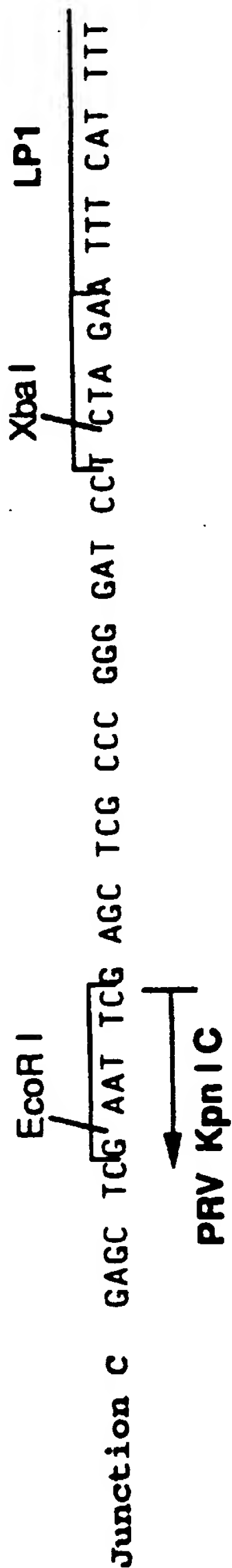
789-41.27
SPV-053



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PCT/US97/12212

FIGURE 14C



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PCT/US97/12212

FIGURE 14D

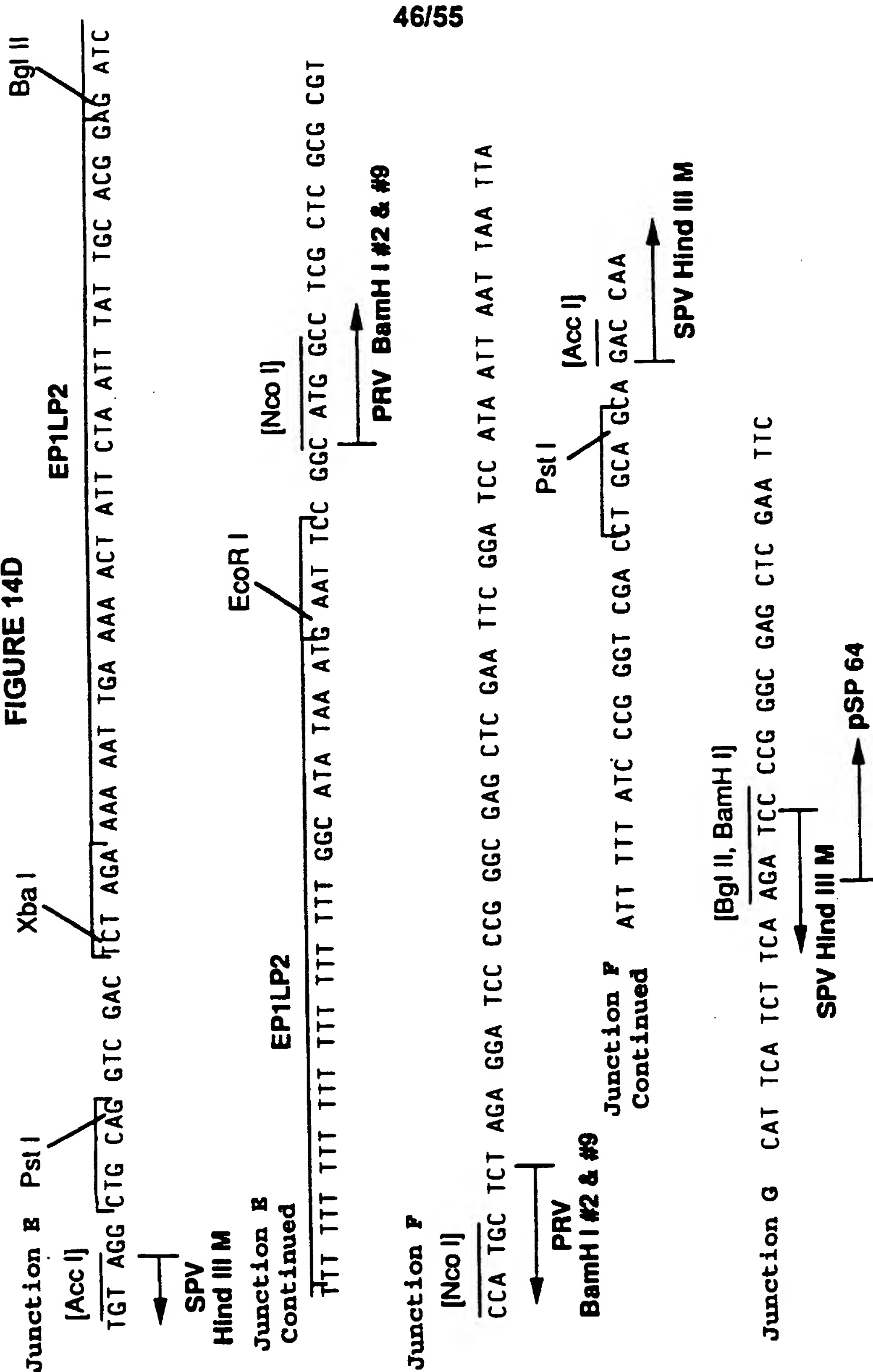
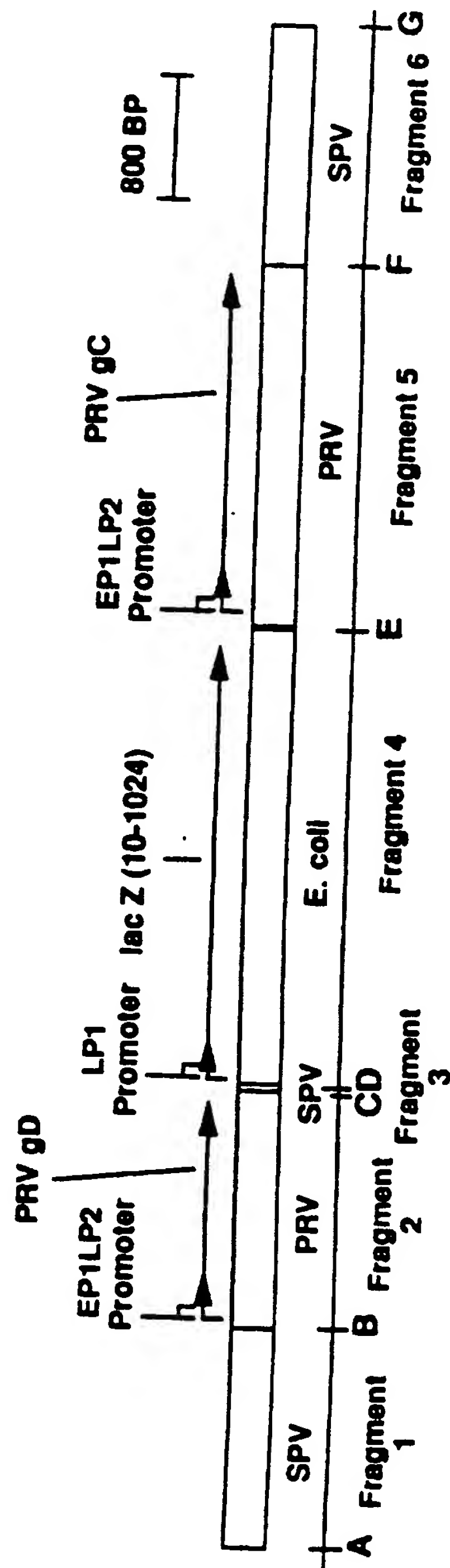


FIGURE 15A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III-BamH I	~2972 BP
Fragment 1	SPV Hind III M	Bgl II-Acc I	~1484 BP
Fragment 2	PRV BamHI #7	EcoR I-Stu I	~1552 BP
Fragment 3	SPV Hind III M	Acc I-Nde I	~48 BP
Fragment 4	pJF751	Bam HI-Pvu II	~3010 BP
Fragment 5	PRV Bam H I #2&9	Nco I-Nco I	~2378 BP
Fragment 6	SPV Hind III M	Nde I-Hind III	~1560 BP



Junction A

[Bgl II, BamHI]

GCC CGG GGA TCT TGA AGA TGA ATG CAT
 └──────────┬──────────┘
 SPV Hind III M

pSP64-

Junction B [Acc II]

Ps11

247193


ATT TGG TCT GCT GCA GGT CGA CTC TAG AAA AAA TIG AAA AAC TAT TCT AAT TTA TTG CAC

SPV Hind III M

Junction B continued


2d71p3

EcoRI

GGA GAT CTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT
GAA TTC | GCT CGC AGC GCT

PRV Bam HI #7

PRV Bam HI #7

[Siv II]

Junction C GCG TGC ACC ACG AGG GAC TCT AGA GGA TCC ATA ATT AAT TAA TTA ATT TTT

PRV Bam HI #7

PRV Bam HI #7

Psi

[Acc I]

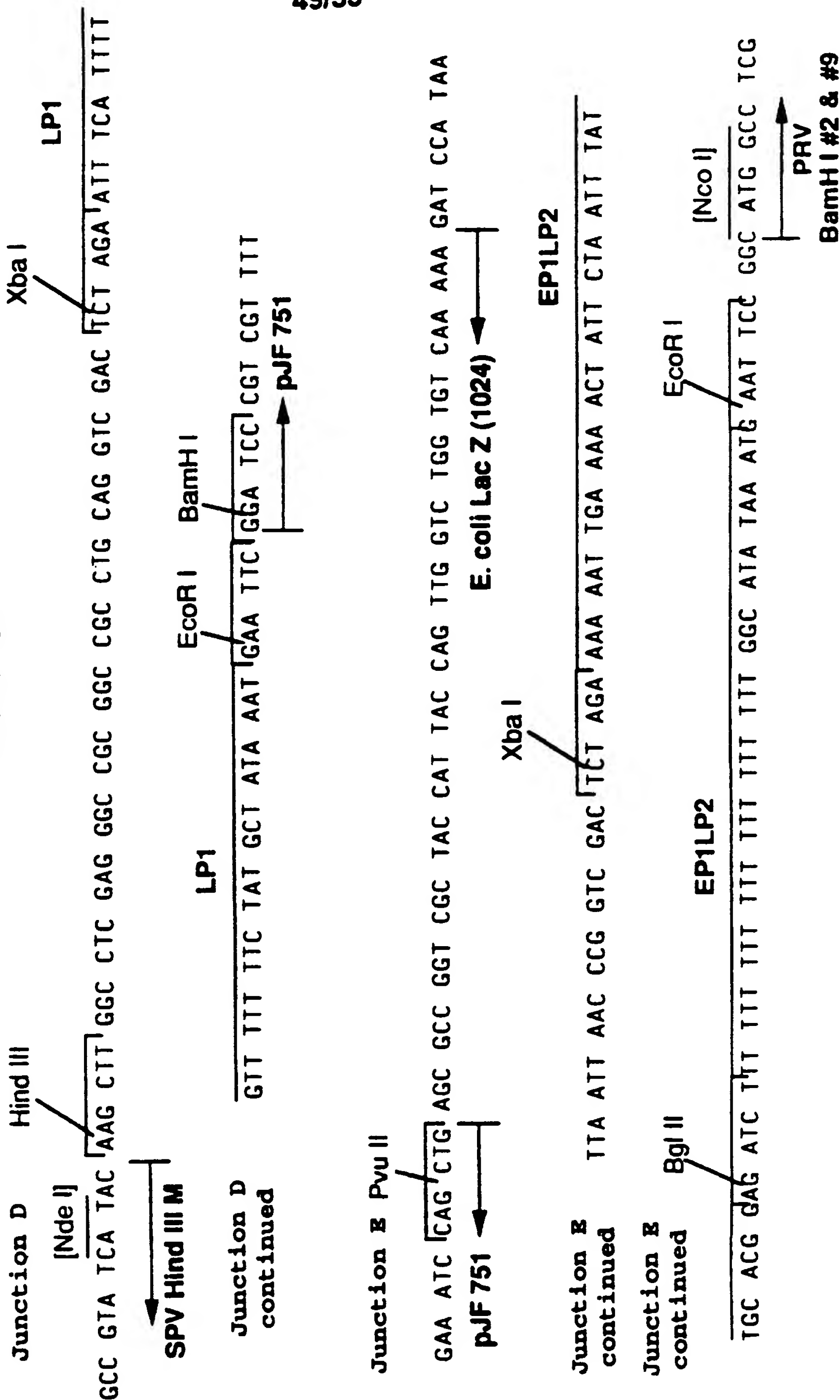
Junction C
continued

ATC ↓CG GGT CGA ↓CCT GCA GCC TAC ATG GAA ATC TAC CAG

SPV Hind III M

SPV Hind III M

FIGURE 15C

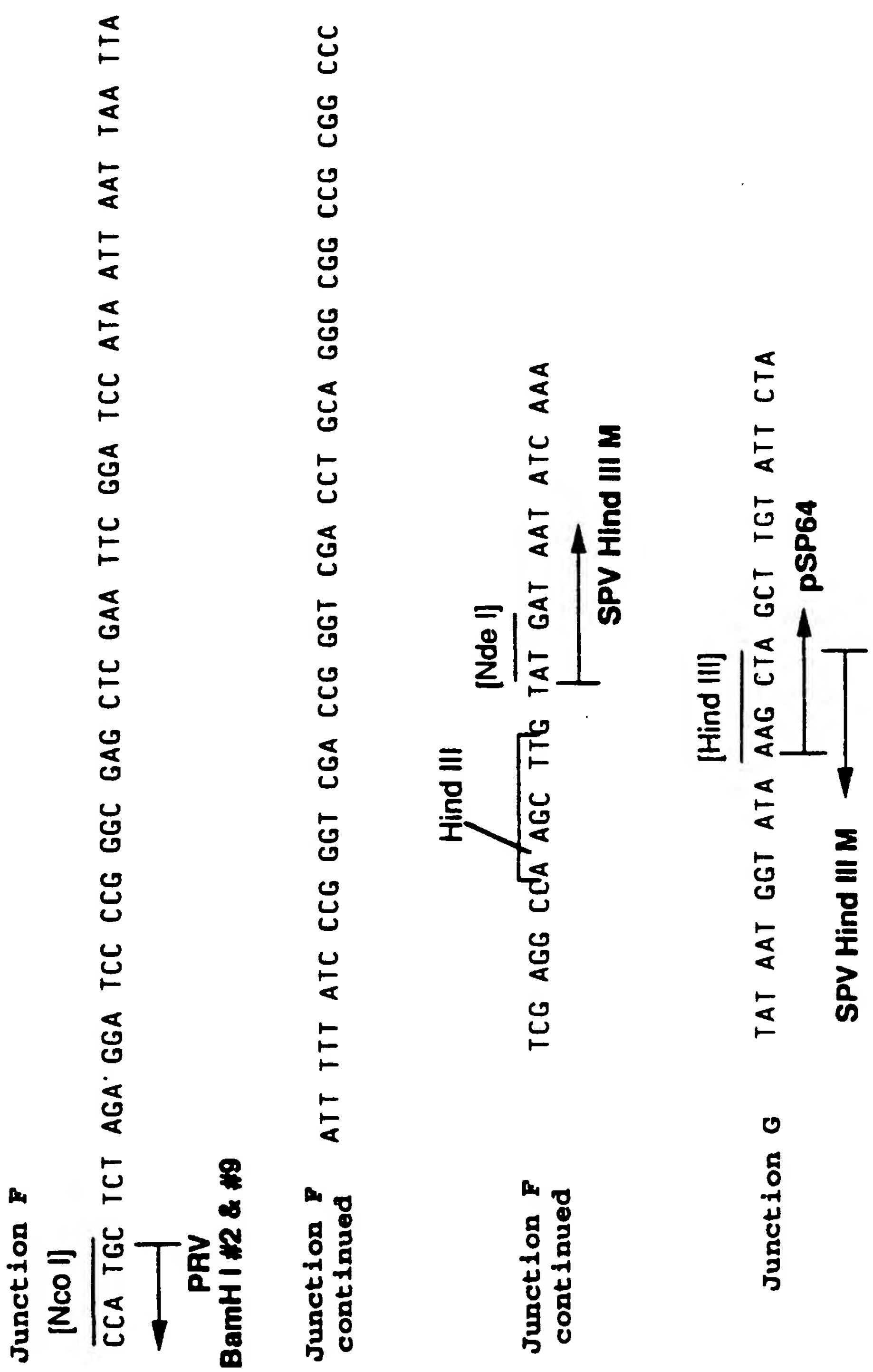


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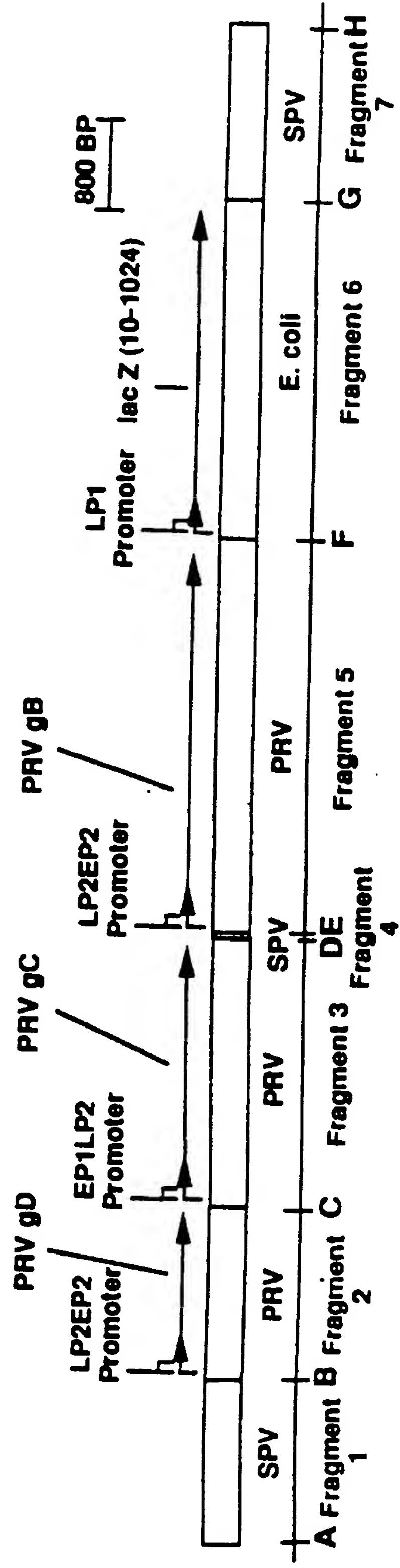
FIGURE 15D



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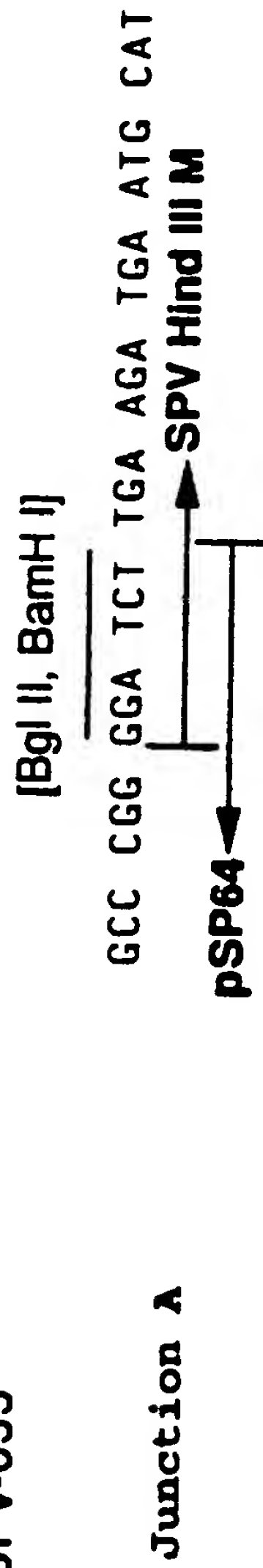
FIGURE 16A

DNA	Origin	Sites	Size
Vector	pSP64	Hind III-BamH I	~2972 BP
Fragment 1	SPV Hind III M	Bgl II-Acc I	~1484 BP
Fragment 2	PRV BamHI #7	EcoR I-Stu I	~1552 BP
Fragment 3	PRV BamHI #2		Nco I-Nco I	~2378 BP
Fragment 4	SPV Hind III M	Acc I-Nde I	~48 BP
Fragment 5	PRV Kpn I C	Sma I-EcoR I	~3500 BP
Fragment 6	pJF751	Bam HI-Pvu II	~3010 BP
Fragment 7	SPV Hind III M	Nde I-Hind III	~1560 BP

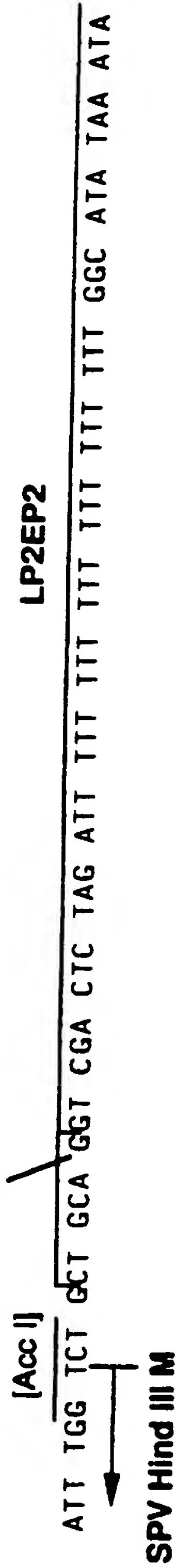


789-41.73
SPV-055

FIGURE 16B



Junction B



Junction B
continued

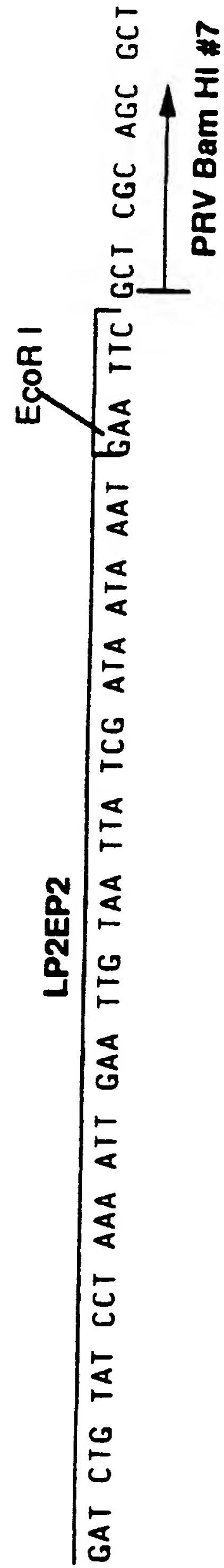
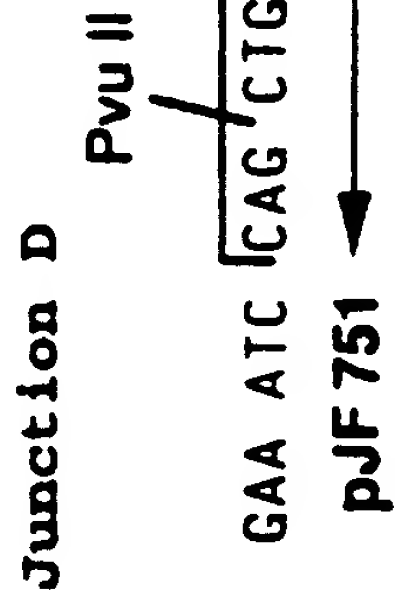
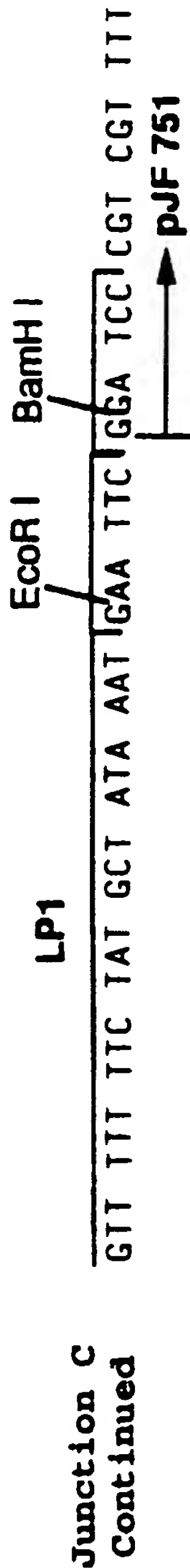
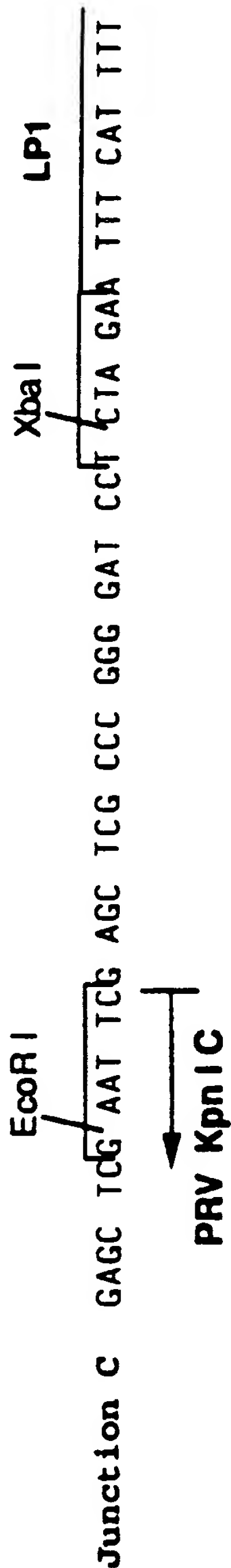
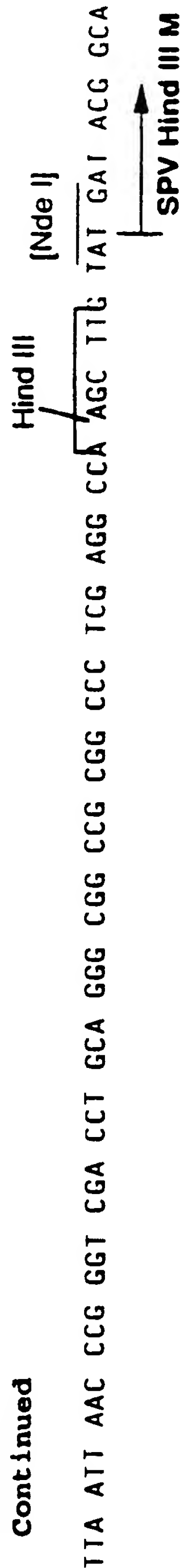


FIGURE 16C



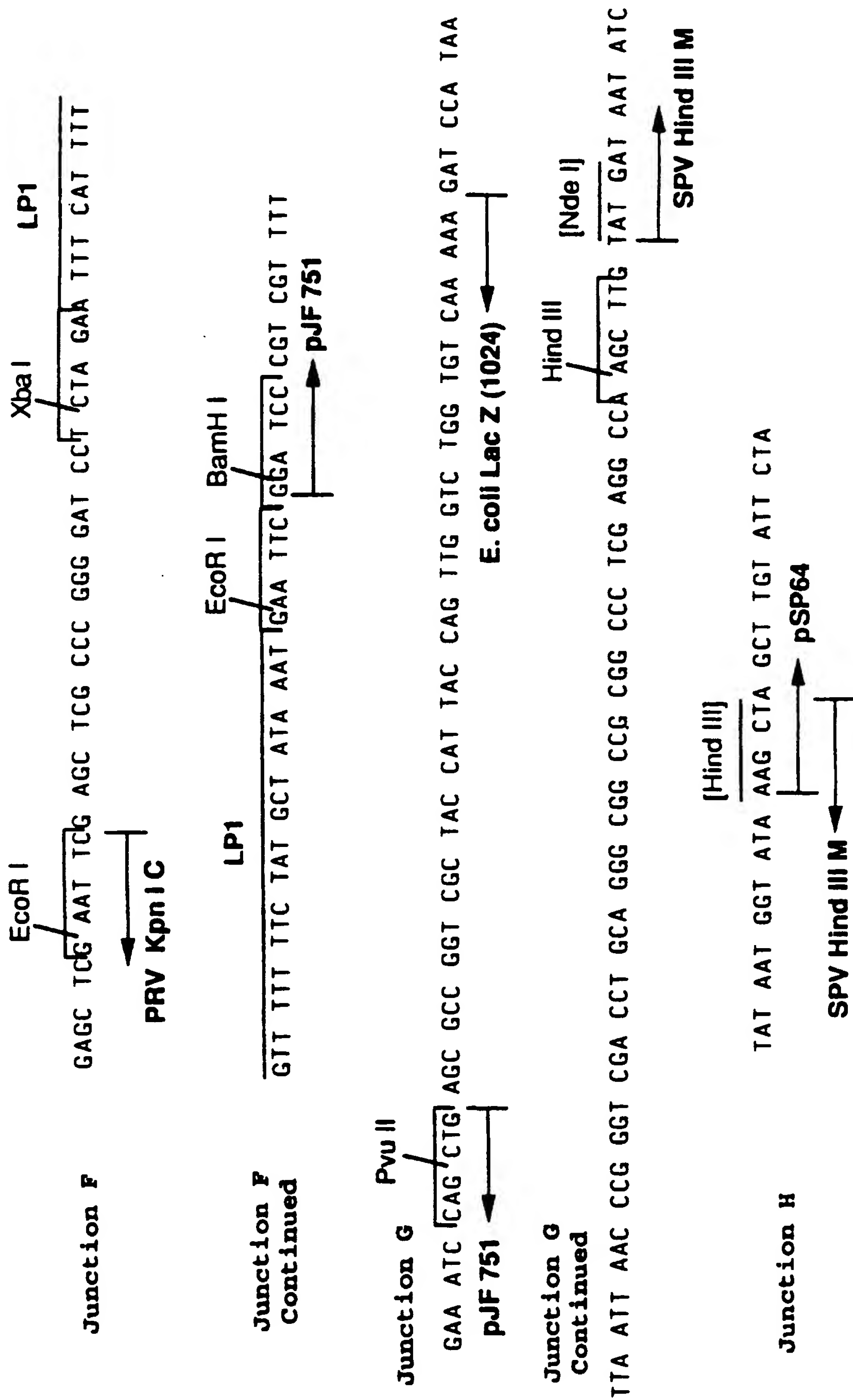
Junction D Continued



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FIGURE 16E



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/12212

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 7/01, 15/86; A61K 39/275, 39/295

US CL : 435/235.1, 320.1; 424/199.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/235.1, 320.1; 424/199.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, WPIDS, MEDLINE, CAB. Search terms: swinepox, swine pox?, suipox?, interferon, cytokines, cytokine, interluk?, bovine, recombinant, vector?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,382,425 A (COCHRAN et al) 17 January 1995, see entire document, especially claims 1-20.	1-35
Y	RAMSHAW et al. Cytokine expression by recombinant viruses-a new vaccine strategy. TIBTECH. December 1992, Vol. 10, pages 424-426, see entire document.	11, 21
Y	MASSUNG et al. The Molecular Biology of Swinepox Virus. Virology. 1991, Vol. 180, pages 347-354, see entire document.	1-7, 12, 15, 21, 34, 35

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	* T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
* A* document defining the general state of the art which is not considered to be of particular relevance	* X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
* B* earlier document published on or after the international filing date	* Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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International application No.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MASSUNG et al. DNA Sequence Analysis of Conserved and Unique Regions of Swinepox Virus: Identification of Genetic Elements Supporting Phenotypic Observations Including a Novel G protein-Coupled Receptor Homologue. Virology. 1993, Vol. 19, pages 511-528, see entire document.	1-7, 12, 15, 21, 34, 35
Y	FOLEY et al. Recombinant DNA Technology I. Annals of The New York Academy of Sciences. New York, New York: The New York Academy of Sciences. 1991, Vol. 646, pages 220-222, see entire document, especially Abstract, and Results.	34-35